THE MASTERWORK OF JULES TISSOT

ENGLISH EDITION

VOLUME I

This translation of J. Tissot's 1926 work 'Constitution des Organismes'
is based on the original French edition, which is in the public domain.
Translated by **Someone for Everyone** in 2025

Cover picture provided by ChatGPT

Dedication

To all who search for meaning
in the smallest of things, and to
the unseen forces that give life
its Beginning and its End

Translator's Note

This book is translation of Jules Tissot's ''Constitution des organismes'' (1926).
I could not read in French, neither I could find any information in English.
There was only one way to read all 3 volumes of Tissot's work–I had to
translate them myself (with the help of Google translator);
paragraph by paragraph, page by page.

The translation has been done. What now? To keep it in my computer?
I believe that there are others out there who has never heard
of Jules Tissot and his great research work.

Jules Tissot deserves recognition!

I did this translation for myself but now, when it's finished…
I releasing it for everyone to read. English is not my language,
So, don't judge me for grammar mistakes. I did as best as I could.

The numbered line-breaks match the page numbers
of the original work if the reader wants to check translation.
The text/wording is left without editing.

I'm not a book writer, I'm not a book editor, I'm not a scientist, I'm not a doctor.

I'm the one who wants to understand. I want to know.
Do you?

Although every precaution has been taken in the preparation of this book,
the author/translator assume no responsibility for errors or omissions.
Neither is any liability assumed for damages resulting from the use of
information contained herein.

Jules Tissot

(17 February 1870 in Besançon – 17 June 1950 in Paris)

Life

After his bachelor's degree in science, which he obtained in 1890, he continued his studies thanks to a scholarship with Auguste Chauveau[1] (1827-1917), holder of the chair of comparative pathology of the Muséum national d'histoire naturelle de Paris. He defended his science thesis in 1895 (titled Study of survival phenomena in muscles after general death) and a thesis of medicine in 1897 (The Laws of Energy Movement in Muscles in static Voluntary Contraction; new method for studying the physicochemical phenomena of respiration). Appointed preparer of Auguste Chauveau in 1898, then assistant in 1906, Tissot served as interim professor in 1909.

In 1920, he succeeded Louis Lapicque[2] 1866(1866–1952), who had himself replaced A. Chauveau, at the head of the chair of general and comparative physiology. In 1933, he renamed it in general physiology. After an accident, he retired in 1940 while continuing his research.

Work

Together with Ferdinand Laulanie[3] he established measures of energy expenditure that would enable Chauveau to found his laws on muscular work.

As a great experimenter, he perfected many laboratory devices, particularly in relation to respiration (he was particularly interested in chloroform anaesthesia). Thus, in 1904 he invented a closed-circuit spirometer. In 1907 he developed an insulating breathing apparatus for mine rescue teams. In 1915 he worked on adapting this aircraft to military use against combat gas; a first version of the "Tissot device" would be manufactured in number from April 1916 (large model); a second (small model) version was adopted in March 1917. Tissot also invents an apparatus intended to renew the air of the submarines.

His research in microbiology led him to issue, in 1926, a theory close to the theses of Antoine Béchamp (1816–1908) on the microzymas which would in fact be moulds (or in a broader sense, fungi and microscopic yeasts, current objects of mycology). According to Tissot, plant and animal organisms are not exactly made up of cells, but normal “organic moulds” that form the tissue weft. These moulds can turn into “pathogenic moulds” by vegetation, so pathogenic bacteria are mycelium segmentations. As Tissot interprets numerous physiological phenomena (immunological reactions, coagulation, phagocytosis, haematopoiesis, etc.) in mycological terms, all the physiology tends to be reduced to the reproduction physiology of fungi.

Similarly, all infectious diseases are reduced to organic moulds becoming pathogenic. The tuberculosis agent is abnormal cladding in mitochondria (normal elements of organic moulds). Tissot provides a complete picture of his findings, which can be seen, for example, that the typhoid agent has as its original source an organic mold of maize, that of oat typhus, the diphtheria of the barley, the measles of the lettuce, the rage of the carrot, the tetanus of the potato, and the syphilis of the monkey.

This theory, which involves certain aspects of the transmission of diseases discovered by Louis Pasteur (1822-1895), encounters only silence and indifference in scientific circles, with the exception, however, of a benevolent account published in 1937. Considering that there is a conspiracy against him of silence, orchestrated by the management of the Pasteur Institute, he founded in 1948 an association, the League of Health and Liberty, whose members refused to impose vaccinations by affirming their danger. Tissot proposes to advantageously replace them by the removal of cereal meal from children's food. While its name is often cited by unconventional health currents opposed to vaccinations, its exact theory is never specified.

Source: https://fr.wikipedia.org/wiki/Jules_Tissot

1. *Jean-Baptiste Auguste Chauveau (1827–1917) was a French veterinarian, physician, physiologist and anatomist.*
2. *Louis Édouard Lapicque (1866-1952) was a French physician, physiologist and anthropologist.*

3. *Ferdinand Laulanié (1850-1906) is a French veterinarian, professor of animal physiology and director of the National Veterinary School of Toulouse.*

Preface

This book is more than an act of preservation; it is an invitation to scholarly reconsideration. Nearly a century after its appearance, this study has remained largely inaccessible, overshadowed by the rapid developments in microbiology, genetics, and ecology that followed. Yet to consign Tissot's work to obscurity would be to overlook a document of intellectual ambition and historical significance.

When a work is forgotten, it does not always mean it has lost its value. Sometimes it simply means that time has moved on too quickly, or that the voice of an author was quieter than the currents of his age. Such is the case with Jules Tissot and his study on *Constitution des organismes*, published in three volumes in 1926, 1936 and 1946.

Jules Tissot's work, long hidden in the shadows of history, deserves to be read, questioned, and remembered. By giving it a second life, I hope to share the depth of thought it contains and the questions it still provokes. May every reader find in these pages not only knowledge, but also inspiration to look at life with new eyes.

At its heart, this study is an ambitious attempt to understand life itself. Animals, plants, molds, and diseases were, in his analysis, not isolated phenomena but interdependent expressions of organic processes. In emphasizing the formative as well as the destructive role of molds and microscopic agents, he anticipated perspectives that modern ecology and microbiology would later confirm: that decay, transformation, and renewal are not peripheral to life, but central to it.

To modern readers, some of his conclusions may appear speculative, even outdated. Yet this is precisely why the work deserves a second life: because it reminds us of a time when science was still close to philosophy, when bold questions were as important as precise answers.

We live in a century that urgently needs to remember the interconnectedness of life. At a moment when global challenges —environmental, medical, and philosophical — press upon us, Tissot's voice joins a chorus reminding us that the foundations of life are shared, fragile, and deeply bound together. His research is not just an artefact of the past; it is an invitation to think, to question, and to wonder.

By placing the first volume into the hands of readers, I hope to give Jules Tissot the audience he did not fully receive in his own time. I believe that everyone who opens these pages will find themselves drawn into questions that remain urgent: What is life? Where does it begin? How do the smallest forces shape the whole?

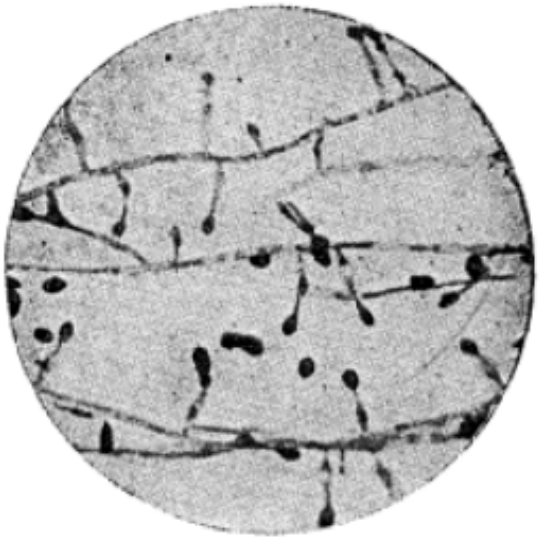
The value of *Constitution des organismes* lies not only in its content, but in its example:
a demonstration of the intellectual daring required to treat life as a whole.

This preface was written in dialogue with ChatGPT — a reminder that voices, whether human or artificial, may help us rediscover what has been forgotten.

J. TISSOT
PROFESSEUR OE PHYSIOLOGIE GÉNÉRALE AU MUSEUM
D'HISTOIRE NATURELLE

CONSTITUTION DES ORGANISMES
ANIMAUX ET VÉGÉTAUX

CAUSES DES MALADIES
QUI LES ATTEIGNENT



LA SYPHILIS

PARIS

AU LABORATOIRE DE PHYSIOLOGIE GÉNÉRALE DU MUSÉUM
D'HISTOIRE NATURELLE
RUE CUVIER, 7

1926

J. TISSOT

PROFESSOR OF GENERAL PHYSIOLOGY AT THE MUSEUM
OF NATURAL HISTORY

—

CONSTITUTION OF ORGANISMS

ANIMALS AND PLANTS

CAUSES OF DISEASES

THAT AFFECT THEM

PARIS

AT THE GENERAL PHYSIOLOGY LABORATORY OF THE MUSEUM

OF NATURAL HISTORY

7 RUE CUVIER

1926

I DEDICATE THIS BOOK TO

DR. ED. LAVAL

AND TO

DR. DESMARET

PROFESSOR AT THE PARIS FACULTY OF MEDICINE

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* * *

Introduction

The studies that are the subject of this work began in February 1922; I must clarify this date by explaining why none of the new knowledge presented here has yet been published in scientific collections.

At the very beginning of this research were observations demonstrating that certain categories of leukocytes do not belong to the animal organism, and that the phenomenon of Phagocytosis is not a phenomenon of defence of the organism, but the phenomenon which results in the very formation of white blood cells; the same observations led to the isolation of the agent constituting a cancerous tumor, both in the form of a bacterial culture and in the form of a mold.

Should I make these facts known through publications in scientific collections? I realized that such a publication would first of all lead to sparking heated controversies, the first result of which would have been a disturbance detrimental to the continuation of my research, due to the need to respond to the criticisms opposed to my observations. These criticisms would certainly have been partly justified, because many points of my observations still remained unexplained.

For these reasons, I gave up publication at this time and continued the research. New observations, always requiring new determinations, showed me the impossibility of a publication relating to facts whose real significance never appeared until much later.

Furthermore, these phenomena were of such importance that it was unacceptable to publish them before decisive proof and demonstrations had been provided. This result is now established, but could only be obtained after three years.

It was only then that the research led to results with important practical applications concerning the treatment and prophylaxis of diseases. The publication of these results therefore became necessary and takes precedence over all other considerations. The new knowledge provided in this work is now based on solid foundations and mutually supports one another.

This research required a great deal of research material. I must thank here my colleagues at the Pasteur Institute, the Faculty of Medicine, and the hospital physicians who helped me, often with inexhaustible kindness, by providing this research material; These thanks go especially to Doctor Laval and Professor Desmaret.

It was with them that the cancer cure attempts that preceded the research that is the subject of this work were initially pursued; later, these were outlined through the study of cancerous tumors that they made available to me. I also owe thanks to Doctor Valleteau de Mouillac, of Cherbourg, who sent me numerous materials with the help of which I was

able to conduct highly interesting studies on various diseases, including tuberculosis, cancer, tetanus, smallpox, malaria, etc.

From the very beginning of this research, I recognized the need to produce, as a demonstration, a photographic representation of the objects or phenomena observed. I was unable to do this and had to supplement it first with a few drawings; later, I created a simple setup allowing the rapid production of a large number of photographs of microscopic preparations. I was thus able, as can be seen in the numerous plates accompanying the text, to provide a photographic representation of almost everything seen and described. These photographs constitute testimonies and demonstrations that will allow the reader to follow the descriptions as well as they would on a microscopic preparation.

For convenience, these plates have been left out of the text and free, so that they can be compared side by side, which is often necessary.

This book addresses a wide variety of questions relating to Histology, Physiology, Bacteriology, and Pathology, and of interest to animal and plant organisms. This diversity was not sought; it was observation, not a prior program, that led this work along the path outlined below.

Studying such a large number of varied questions, it was impossible for me to provide a historical and bibliographical overview for each of them because, to address each question in this manner, I would have had to triple the volume of this work and significantly delay its publication. The reader will realize that the purpose of this research was more to identify general principles than to address specific questions.

The publication of this work was greatly facilitated by the advice of Mr. Audin, editor, and by the steps he took to bring it to fruition promptly; I offer him my thanks here for his friendly and valuable collaboration.

December 1, 1925.

ORIGIN AND NATURAL DEVELOPMENT OF RESEARCH

This research originated from observations made during a microscopic study of cancerous tissue. The study of the nature of cancer, based on published work on this subject, led me to admit, as others had already done, that this disease is not of parasitic origin and that it results from a deviation in the properties of certain cells in the body, manifesting itself through exaggerated multiplication activity.

Based on this idea, I thought that this abnormal cellular multiplication could at least be stopped, in cancer patients, by the action of antibodies obtained by injecting animals with albuminoid substances from the nucleus of cancerous cells.

The experimental research that I had instituted on these bases and that I was able to carry out under exceptionally favorable conditions, thanks to the help given to me by Doctor Laval and Professor Desmaret, is set out in the "Cancer" section. During this research, I deemed it necessary, at a certain point, to undertake a microscopic study of the cancerous cell.

To this end, my friend Laval provided me with some cancerous tumors. The first one he sent me was the origin of the research presented in this work. It was a remarkable tumor, a breast cancer as big as a small tangerine, but obviously one of the most serious, because the lymph nodes in the groin already constituted a mass twice as large as the breast tumor.

Microscopic examination of living cancerous tissue. - This examination took place on preparations obtained by lightly scraping the surface of a cross-section of the tumor and placing the resulting liquid between a slide and a cover-slip after staining with a little methylene or dahlia blue. The preparations thus made contained the following elements:

1. Spherical masses with granular contents having all the appearance of white blood cells or leukocytes, but carried at the end of a stalk.

2. Other bodies appearing of the same nature, but spindle-shaped and also stalked.

3. Ovoid, transparent cells, of the shape and size of the plague bacillus, and very often containing either a very small round globule at one of its ends, or a globule at each end. These globules, which were colored dark blue by methylene blue, were animated by Brownian motion inside the cells. Other similar, free globules, also animated by Brownian motion, moved briskly in the field of the preparation. The ovoid cells, containing one or two of these globules, also moved with a lateral rocking of their ends, analogous

to that of a boat whose oarsman alternately acts on both oars. Many cells were devoid of globules and these were immobile. It was therefore evident that the apparent mobility of the cells was communicated to them by the Brownian motion of the small spherical globules. This was corroborated by the fact that the debris of any element seen in the preparation began to move as soon as a globule attached itself to them. Finally, at a given moment, and several times, I saw the globule that was moving at the end of a cell quickly escape and the cell become immobile.

4. Numerous rods, similar to the typhus bacillus, staining strongly with methylene blue and mobile like the previous elements when a globule attached itself to their end.

5. Spherical globules of various sizes, transparent, not stained with methylene blue, some of which carried inside them, at one end of the diameter, a thin globule stained dark blue, or two globules, one at each end of the same diameter.

6. Mono- and polymorphonuclear leukocytes.

7. Elements of odd shape, branched or not, which I could not connect to any known element. Despite the numerous failures of attempts to culture the problematic agent of cancer, I attempted cultures in broth and on agar.

The next day, the broth culture was already developed; examining it between slide and cover-slip at high magnification and after staining with methylene blue, I found the rod-shaped bacillus as well as the ovoid bacillus similar to the plague bacillus, and, in addition, long filaments which segmented, giving rise to elements rigorously similar to the rod-shaped bacillus.

I also found the spherical globules animated by Brownian movement and I saw that they carried with them the elements to which they came to attach themselves.

I examined a preparation of this culture all day, because what I saw was remarkable. Certain ovoid cells, animated by apparent movements by the spherical basophilic globules, moved, for example, towards a cluster of four or five (or more) other similar cells whose mass seemed to attract them and stuck to it.

Rod-shaped bacilli also merged with this mass, then the whole fused in such a way that the original elements were no longer distinguishable and only a granular mass with a single irregular or spherical nucleus was visible.

The body thus formed was exactly like a mononuclear leukocyte. Looking in the preparation, I noted masses at all stages of formation and saw some that had reached the perfect mononuclear leukocyte form, others that had reached the polynuclear form. I noticed that, in certain masses, there were bacilli in the process of fusing with the

already formed nucleus; I say in the process of fusing because part of the bacillus was still clearly visible outside the nucleus, the rest already being included.

This phenomenon of inclusion, then fusion of the bacilli, was clearly *phagocytosis.*

Referring to fresh preparations of the cancerous tumor that I carefully preserved in the icebox, I found there a multitude of similar free bodies, mono-or polymorphonuclear, that I had t*aken the day before for leukocytes.*

It was therefore likely that these elements had the same origin and were of the same nature as those whose formation in vitro by agglomeration and fusion of bacterial elements I had just observed.

In this new study of the tumor, I noticed very fine arborizations consisting of one or more branched filaments, barely tinted by methylene blue or even uncolored, whose branches terminated in rounded corpuscles. These arborizations resembled the spore-forming hyphae of *Trichophyton* and *Microsporum.*

By thus alternately referring the observation of the broth culture to the cancerous tumor, I was convinced, after three or four days of examination, that all the polymorphic elements observed corresponded to successive stages in the evolution of a single parasitic agent that seemed to me to be related to the genera *Trichophyton* or *Microsporum.*

Other cancerous tumors were sent to me by Doctor Laval. For each of them, I succeeded in obtaining cultures and making identical observations. From the observations made, I therefore had to conclude:

1. That bacteria have no individuality and are only the segmenting elements of more complex organisms of the hyphomycete group.

2. That in a bacterial culture, by agglomeration of the elements, bodies identical to the normal leukocytes of the animal organism are formed.

3. That the normal and constant lymphocytes of the animal organism are elements formed by bacterial agglomeration, during the evolution of hyphomycetes infecting the organism.

4. That certain pedunculated leukocyte elements, existing in cancer preparations, must have been terminal or lateral formations of the infecting hyphomycete.

5. That phagocytosis was an error and that what had been taken for the destruction of bacilli by lymphocytes was their normal mode of formation.

6. That all known bacterial species are probably only the segmentation products of hyphomycetes.

If these conclusions were correct, it should therefore be possible to return a bacterial culture to its original hyphomycete state by appropriate culture procedures.

These various points were studied in the following manner:

1. I obtained a collection of microbial species which was given to me by Professor Bezançon's laboratory at the Faculty of Medicine, and I studied their development in broth and on agar from twelve to twelve hours.

2. I inoculated all the bacterial species that had been given to me onto various solid media.

3. I inoculated onto similar media the bacterial cultures that I had obtained from the various cancerous tumors that my friend Laval had provided me, with a view to arriving, if possible, at a vegetation similar to that of *Trichophyton* and *Microsporum.* At the same time, I studied the changes in shape of the cultures under the microscope.

4. I made cultures of *Trichophyton* and studied their changes in shape.

5. I made a microscopic study of the various tissues of the organism: brain, liver, spleen, etc., with a view to researching the origin of the normal leukocyte forms.

With the cultures in liquid media, the results were fairly quickly conclusive: here they are:

1. In broth cultures, microbial proliferation occurs through the formation of agglomeration masses or germinative masses emitting mycelial filaments, the segmentation of which gives rise to bacterial elements; fine basophilic granules animated by Brownian movements are always formed there. But it was impossible for me to observe the formation of a persistent mycelium. As the cultures age, they form agglomeration masses of varying size and appearance, which cease to evolve.

2. In potato or carrot cultures, we observe the formation of a lasting mycelial vegetation (at least for a certain time) of spherical pedunculated bodies(chlamydospores) similar to white blood cells, and *sporiferous hyphae* similar to those of *Trichophyton*; the mycelial ramifications segment to form bacteria of various types.

The examination that I made, at that time, of a veil of Koch's bacillus on broth that Doctor Kuss had given me, showed me that this veil was made up of an extremely ramified mycelium bearing fruiting bodies that I could not identify because of their thickening.

3. It normally exists, constantly, in the brain, liver, spleen and other tissues, in humans, horses, oxen, donkeys, sheep, goat, pig, rabbit, guinea pig, rat, mouse, mole, etc., contain mold elements (mycelial and fruiting bodies).

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The apparently healthy animals therefore appeared to me, at that time, all to be affected by various, non-apparent mycoses. Their organs contain a large quantity of granular formations that are spherical, pyriform, spindle-shaped, etc., all stalked and still adherent to the mycelium that formed them.

These observations led me to study the development of molds with a view to comparing their vegetative and fruiting organs with the elements observed in the tissues.

I didn't have to look far for a subject to study.

Some coffee, which I had left in the laboratory for several days, had become covered with mold on the surface. This mold examined was pure *Penicilium (Penicilium candidum* according to Mr. Vincent, a preparer in Professor Mangin's Cryptogamy Laboratory). I inoculated it on glycosylated peptone broth. After two or three days, this very cloudy broth was covered with a veil of *Penicilium* and gave off a foul odor. I made a microscopic preparation of the broth and examined it.

I was very surprised to find the most diverse bacterial forms there, notably those of the *typhus bacillus*, those of the *coli,* of the *diphtheria bacillus*, and forms resembling Koch's bacillus. Thinking that I had been able to reproduce foreign germs in the broth, I took *Penicilium* spores from the surface of the veil with the greatest care, barely touching it, and I made a second subculture of these spores with the culture obtained; even with all these precautions, I observed the same bacillary forms.

I quickly had proof that they did indeed belong to this single *Penicilium*, because, upon examining the floating mycelium, I noted that it emitted ramifications of different shapes, whose s*egmental elements, still undissociated, had exactly the shape of the bacterial elements observed in the broth.*

Moreover, the vegetation of the mold studied showed, in the mycelial part floating on the broth, formations similar to those I had observed in the organs of animals. These latter were therefore indeed elements of molds vegetating in the animal organism. These observations constituted proof that the known bacterial species are segmentation products of the mycelium of higher molds, *Penicilium, Aspergillus, Mucor*, etc.

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If this conclusion were correct, it should be possible, starting from laboratory bacterial cultures, to reconstruct the original molds from which they originate and thus provide new, decisive proof of the nature and origin of bacteria.

Reconstitution of pathogenic molds in the state of hyphomycetes by bacterial cultures. - This proof was given to me by a culture of typhus bacillus that I had placed on a fragment of refractory earth slightly moistened with broth; it gave rise to an aerial mold which, examined, was a *Penicilium* of the *Citromyces* type.

There was no doubt that this *Citromyces* was indeed a hyphomycete form of the typhus bacillus because, the spores being replanted for several generations in a row on carrot, typhus bacillus was found on the surface of the latter in the intricacy of the mycelial branches. At the same time, other bacterial species, coli, diphtheria bacillus, anthrax bacillus, gonococcus, were transformed into their aerial mold.

Examining a two-month-old diphtheria culture on agar from the Pasteur Institute, I noted, in this very culture, as soon as I received it, the *presence of typical Citromyces gonidiophores bearing their bouquets of sterigmates.*

For some time, I had been collecting pathological products in an attempt to obtain molds by this route.

By culturing the lymph extracted from a young excised chancre, I obtained the pathogenic mold of Syphilis. I had no reason to doubt this one either, for, examining its mycelium, I saw from the first examination that it was covered with an innumerable quantity of fine spiral filaments that were spirochetes. This taught me that spirillae are not protozoa, but spiral appendages of the mold mycelium.

By varying the culture media, I thus obtained the hyphomycete form of most known bacterial species. The examination of these molds and the study of their development provides an important result concerning the determination of pathogenic agents, causes of contagious diseases. The pathogenic elements designated spirillae, spirochaetes, are not the agents of the disease themselves, but only spiral appendages with which the mycelium of the pathogenic mold is covered. They are not protozoa. *Trypanosomes* are no more:

This fact was demonstrated by the isolation of the pathogenic mold of sleeping sickness. *Trypanosomes* are mycelial formations that appear at the end of the first mycelial filaments formed by the germinal masses of the pathogenic agent.

Isolation of pathogenic molds from diseases with unknown germs. - From these observations it resulted that the pathogenic agents of diseases with known germs are not, in reality, the bacterial species described, but the molds from which they originate. It therefore seemed certain that the pathogenic agents of diseases with unknown germs are also molds.

By culturing the blood, lymph, and crusts of pustules, cerebrospinal fluid, etc., of patients, I succeeded in isolating the pathogenic molds of the following diseases: measles, scarlet fever, chickenpox, vaccinia, smallpox, rabies, malaria, typhus, etc.

The isolation of the malaria agent showed that it has nothing in common with a hematozoan; Laveran's hematozoan is a spore or germinative mass of the malaria mold.

The isolation of these pathogenic molds, as well as the transformation of bacterial cultures into hyphomycetes, was not as simple as one might think. This research was indeed made very delicate by an observation that constituted, at one point, an almost insurmountable difficulty.

In fact, in quite a few cases, I obtained two different forms of mold from the same original bacterial culture, a *Penicilium* and a *Sporothricum*, for example.

With a culture of anthrax originating from Mr. Bezançon's laboratory, I obtained, on the one hand, an *aspergilloid Penicilium* and, on another medium, an *Aspergillus*.

On two tuberculosis cultures given to me by Mr. Legroux of the Pasteur Institute, I obtained, with one, an *Aspergillus* and a *Penicilium*, with the other, a *Penicilium* and a *Monilia.*

Applying current mycological principles, I was forced to conclude that a *Sporothricum* and a *Penicilium* could not come from the same culture, and that one of the two molds must inevitably result from contamination.

Unable to perceive the cause of these facts, I looked for other examples by closely monitoring the inoculations and carefully preserved all forms suspected of contamination.

Rapid production of pathogenic molds by passage of the culture into the animal organism. - The process I used for the direct transformation of bacterial cultures on more or less humid solid media and at room temperature is long. Often, the mold is only obtained after several varied and unsuccessful attempts and after several months.

I had observed that the hyphomycete form of a pathogenic agent existing in a bacterial culture is obtained all the more quickly if the latter contains larger agglomeration or germinative masses.

However, in animals, a large number of germinative masses (leukocytes), often very large, and mycelial elements develop very quickly after the injection of a bacterial culture.

This observation led me to try, in order to quickly obtain the forms of hyphomycetes, and especially those that I had been unable to obtain directly, mainly glanders and plague, to first inject the pure culture into an animal (under the skin of the paw), then to remove the inguinal lymph nodes, *which enlarge as they fill with the vegetation from the injected culture*, and to inoculate the product of the crushed lymph nodes. The result was most conclusive. I also saw that the crushed spleen, liver, and lung also provide the mold of this agent.

It generally appears on the cultures from the third to the sixth day, most often on the fourth day. I verified the accuracy of the process for all the pathogenic molds that I obtained directly from bacterial cultures, that is, in all the cases where I possessed the element of absolute control.

Long experience with this process, which I have used for all the infectious agents discussed in this work, has proven to me that it is safe and rapid. However, at the beginning, it exposed me to easily avoidable errors, the full explanation of which will be given to the reader later in this presentation. Indeed:

In normal guinea pigs, molds always develop on organ pulp cultures. But their development is less rapid and their growth much more discreet than that of recently injected pathogenic molds, which generally thrive on cultures of damaged tissues, particularly on lung tissue cultures.

Finally, the culture of enlarged lymph nodes in the groin or armpit is safe because, in normal guinea pigs, these underdeveloped lymph nodes are almost invisible and generally do not grow or grow very slowly.

Nevertheless, the possibility of confusion with normal mold required:

1. Knowledge of the molds that grow spontaneously on the organs of healthy, normal animals.

2. Control by simultaneous injection of cultures in experiments with guinea pigs and rabbits; the organs of the healthy rabbit spontaneously give rise to molds that differ from those of the guinea pig.

Using this procedure, I was able to quickly obtain the hyphomycete forms of pathogenic agents. By injecting guinea pigs with cultures of tuberculosis, anthrax, etc., I again obtained the same hyphomycete forms that I had already obtained by direct culture. Moreover, I simultaneously obtained, with a culture of anthrax, for example, the two forms *Penicilium* and *Aspergillus* that I had already obtained directly. For other cultures, I obtained three, four, and even five different mold forms.

This did not demonstrate the non-existence of contamination of the original cultures, but it made it highly improbable, because the contamination of a bacterial culture by four or five foreign germs is easily observed by microscopic examination and cannot go unnoticed with careful monitoring.

The facts that previously seemed inconsistent to me were therefore confirmed. It was necessary to deduce that this inconsistency was only apparent, and that the impossibility of explaining the phenomena stemmed from ignorance of an important concept that needed to be investigated.

Knowledge of this concept was provided by the following experiment, which I repeated several times with different species:

Having obtained four different molds by injecting a guinea pig with a bacterial culture, an *Aspergillus*, a *Penicilium*, an *Acremonium*, and a *Sporothricum*, for example, the spores of each of them were injected separately into a guinea pig; each inoculated mold gave rise, in the culture of the animal's organs, to one or more of the other molds obtained from the first guinea pig, in addition to its own form.

This point is developed in detail in the chapter *"Multiplicity of Gonidian Forms."*

On the other hand, careful examination of the gonidian forms of each mold obtained, made it possible to observe forms of transition to the species of the highest rank and to the species of the lower rank. All of these facts meant:

That the pathogenic agent injected into the guinea pig in the bacterial state, that is to say in its simplest form, develops germinative elements there which, transported to another culture medium, undergo progressive transformations *until reaching the highest state: Penicilium or Aspergillus.* This vegetation does not always reach the final stage and may stop at the forms: *Saccharomyces, Sporothricum, Streptothrix, Acremonium, Mucor, Sterigmatocystis, Aspergillus*, etc.

The forms considered until now as different genera, such as the genera *Mucor, Aspergillus, Penicilium*, etc., are therefore only different gonidial states or types in which a mold can appear, and not genera.

Numerous other intermediate forms exist depending on the species, such as *Acremonium, Verticilium, Acaulium*, etc

I also had confirmation of the accuracy of these conclusions by another process; I succeeded in obtaining directly, in vitro, the transformation of one gonidial type of mold into another type, for example, of the *Mucor* type into the *Penicilium* type, of the *Monilia* and *Sporothricum* types into *Penicilium*, etc.

The demonstration of this law of mold evolution allowed me to definitively establish the different gonidial forms of pathogenic molds of diseases with known or unknown germs.

***Normal mycoses of animals.* -** From the demonstration of the mode of formation and the origin of leukocytes, elements of the evolution of molds, I had to conclude, in the current state of biological knowledge, that they do not belong to the animal organism. It resulted from this conclusion that one could predict the constant presence of molds in the organism of healthy animals, whatever the species, since leukocytes are always found there.

The first check of this deduction, already related above, verified it exactly. It was repeated by systematically searching for the vegetative organs of molds, sclerotia, mycelium, and fruiting bodies, in mice, rats, moles, guinea pigs, rabbits, sheep, goats, pigs, cattle, horses, and humans. I easily noted the constant presence of all these mold elements in all these animals and in all their organs: spleen, liver, lungs, brain, in connective tissue, and blood.

This vegetation presented characteristics identical to those of the vegetation of cancerous molds.

The fruiting bodies here take on a form so different from those of aerial molds that it is impossible to identify them at first glance. A systematic search carried out, on the one hand in the animal organism, on the other hand in molds cultivated in different environments and, above all, the in-depth study of the early stages of the development of the latter, allowed me to establish with certainty the equivalence of each of the fruiting forms encountered in the organism and even the reasons for the particular forms they take.

I thus found in the organism the spore-bearing hyphae of trichophytons, the spore-bearing hyphae with sterigmates of *Penicilium*, the chlamydospores of the various species of mold, the mycelium with its different forms, even with its fine tendril-like branches, etc.

In vitro culture, under suitable conditions, of the organs invaded by these molds gave me the aerial form of parasitic molds. These molds are easily obtained with the liver, spleen, brain, with the white layer of leukocytes that covers the blood cells of centrifuged blood.

These observations therefore proved that the elements of molds exist in all organs.

I attributed the presence of these molds to constant and permanent mycoses whose origin must go back, in my mind, to the acute illnesses contracted by all individuals.

I could not doubt the real existence of permanent or very long-lasting mycoses since there is a striking example: syphilis.

But, as the mass of these molds that I considered as parasites is considerable, I was surprised that they did not produce disorders, even apparent ones, since they exist in all healthy individuals. I was also surprised to note that it is always the same molds that contaminate all healthy individuals.

This consideration prompted me to complete my observations, and I resumed the study of all the tissues and organs from the same point of view.

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By examining the connective tissue with the greatest care, I found there all the characteristic elements of mold vegetation, and I was *unable to find a single element of any other nature.*

I observed, in all parts of this tissue, the presence of the element which provides indisputable proof of its nature and origin: the enormous sclerotia from which the large bundles of fibers originate; I was able to see these ramify, follow the fibers which emanate from them and find fixed cells along their path; I also noted that branches, starting from the bundles, carried sporiferous hyphae, chlamydospores and fruiting bodies of various forms. Thus the relationships between the various elements of the connective tissue were established.

Finally, definitive proof, by cultivating on agar, under suitable conditions, pieces of connective tissue taken with all the necessary aseptic precautions, I saw aerial mold appear after three to five days, on certain fragments.

Thus, I arrived at the following conclusion, *which was the most difficult for me to admit,* and which was only admitted after obtaining the most precise and decisive evidence: *connective tissue is formed exclusively, in healthy animals, by the vegetation of molds.*

Should we admit that connective tissue is a tissue of a plant nature? Is it a parasitic tissue? In the current state of biological knowledge, it was impossible to admit that a mold, that is, a fungus, could constitute a part of the animal organism.

However, connective tissue fulfils certain functions in the animal organism, one of which is defined: it is the supporting role that it fulfils in most organs, notably in the liver, the kidneys, the vessels, etc.

How can we reconcile this notion of an obvious and important physiological function of connective tissue with its parasitic origin?

There was an enigma here that hypotheses could not clarify and whose solution could only result from a more complete determinism of the nature of the tissue, its elements

and its relationships with other anatomical elements, notably with those of the noble elements of the organs, elements which are, without a doubt, of an animal nature: such as myelinated nerve fibers, nerve cells of the centers, muscle fibers, etc.

I therefore made a study of most of the organs bearing on both the connective elements and the noble elements. I began this study with that of the nerves.

From my first observations, I noted that Remak fibers are mycelial elements of molds. I saw them bear fruiting bodies similar to those of connective fibers. For the myelinated fibers, it appeared that they are mycelial filaments of molds *having the same form as the mycelial filaments of the various species of the genus Mucor.* I was able to observe:

1. On the myelinated fibers, all the characteristics that belong to the mycelial filaments of a mold of the *Mucor* type.

2. On the mycelial filaments of molds of the *Mucor* type, cultivated in vitro, all the characteristics of myelinated fibers.

I observed in the sciatica the most decisive proof of this nature of myelinated fibers: the existence, at the ends of numerous fibers or their fruiting bodies, of large fruiting bodies, most characteristic of the *Mucor* genus.

The interannular constrictions are the points of partitioning of the mycelium; they typically exist, with their cylinder-axis, in all *Mucor* mycelial filaments.

I investigated whether there are other elements in the nerves, besides Remak fibers, myelin fibers, and connective elements, that could fulfil the physiological functions of the nerves. I found none.

I then moved on to the study of the anatomical elements of the nerve centers, spinal cord, cerebral hemispheres, and cerebellum. I noted that, throughout the nerve centers, all the anatomical elements are made up of mold elements.

I studied with the greatest care the pyramidal cells of the cerebral cortex.

The Purkinje cells of the cerebellum, the multipolar cells of the grey matter of the spinal cord: these are mold elements.

I investigated whether there exist in these centers elements that could not be linked to mold and to which the nervous functions could be attributed. I found none.

Here, the study of the problem had yielded sufficient results for the following conclusion to appear likely:

Since the total mass of nerve centers appears to be made up of one or more molds, these can be neither parasitic nor pathogenic; *they are the anatomical elements of the nerve centers themselves.* But, from this conclusion, it was impossible to provide direct, absolute proof: it is not possible, with our means of investigation, to prove that a multipolar cell or a pyramidal cell is the seat of nervous functions; sections or ablations do not constitute proof, because they affect or can affect elements other than the elements considered.

To obtain this proof, I turned to the muscle, an organ whose functioning *is visible to the eye,* and which therefore allows us to establish a certain relationship between its nature and its functioning.

The microscopic study of the striated muscle fiber was conclusive and provided the definitive explanation. In certain animals, particularly in the snake, it can easily be seen that the muscle fiber is a large bundle of secondary fibers and that each of these is made up of two fibrils, one straight axial, the other spirally wound around the fiber axial. Both bear, along their path, the attributes of fine mold ramifications: spiral appendages and spore-bearing appendages.

Thus the definitive proof was established: *The muscle fiber, an anatomical element fulfilling one of their characteristic functions in animals, motility, is a mold element.* The anatomical elements of nerve centers that I had identified with the mold elements are therefore also elements fulfilling nervous functions.

I obtained the same proof by studying blood corpuscles. These are small mycelial formations of a mold, detached from the organs and launched into the circulation to fulfil a special role. From these facts resulted a conclusion of considerable importance: *The animal organism is constituted by an organized mold.*

It followed that the in vitro culture of a portion of tissue from an animal, under suitable conditions, should give rise to various gonidian forms of the specific mold constituting this animal.

The experiment entirely confirmed this prediction. I easily obtained, by tissue culture, the organic molds of species belonging to all groups: Mammals, Batrachians, Reptiles, Fish, Insects, Worms, Echinoderms, etc. Moreover, I obtained from this organic mold various gonidian forms for each species.

I had already obtained these various gonidial forms by studying the process of transforming bacterial cultures into hyphomycetes by passage through the bodies of animals and culturing their organs.

These are the molds that I saw appearing on in vitro cultures of the organs of healthy animals and *which I wrongly considered to be the agents of permanent mycoses*.

Knowledge of the gonidial forms of organic animal mold makes it possible to avoid confusing them with heterogeneous molds subjected to experimentation, when using the process of transforming pathogenic agents by passage through an animal's body.

The fact that an animal is constituted by the organization of a mold implies that the latter, obtained by culturing its organs in vitro, *is the primitive or ancestral form of this organism,* if, during its evolution, it has not been modified or adulterated by crossing with other molds, or if its chemical constitution, on which its characteristics certainly depend, has not been slowly modified, notably by diet.

Since animal organisms have this origin, it is likely that the same is true for plant organisms and that, in all their tissues or organs, one must find the elements of the specific organic mold that constitutes them; one must also be able to reproduce the ancestral or original mold by culturing any portion of the plant's tissues.

The verification of these deductions in plants was as conclusive as in animals. Plant tissues are made up of an organized mold whose characteristic elements are easily distinguished; by culturing any portion of tissue, one easily obtains, in vitro, the gonidian forms of the ancestral mold.

A new microscopic study of animal and plant tissues, guided by this new knowledge, showed that *cellular individuality does not exist there.*

A liver cell, for example, is not an isolated, distinct element; it is an element formed by a mycelial filament itself coming from other branches and, at the origin of these, from a distant germinative mass; the liver cell cannot live if it is not connected by its formative mycelial filament to the original germinative mass on which it depends; this germinative mass is probably the source or reservoir of the basophilic protoplasm which the liver cell needs to form the numerous zygospores which it contains, and whose purpose is the exercise of the chemical functions of which the liver is the seat.

The mycelial filament connecting the liver cell to the germinative mass is, as in all molds, the channel which serves for the flow of the basophilic protoplasm from the germinative mass to the terminal element; This is the phenomenon that takes place in a simple element of *Mucor* consisting of a germinal mass, the ramified mycelial filament that emanates from it, and the known fruiting balls placed at the terminal end of each branch; we see the flow of basophilic protoplasm from the germinal mass through the mycelial branches to the fruiting bodies. This basophilic protoplasm, in the process

of flowing, is rejected from the element in the form of round spores released when the cap of the fruiting body breaks.

Spores are formed as long as the germinal mass provides basophilic protoplasm; when it is exhausted, *we see that the flow channel empties of basophilic protoplasm*, then withers and dies; the life of the element is over.

There is therefore no independent cellular life. In tissues, a large number of neighboring cells are connected to the same mycelial filaments and to the same germinative mass and all receive from them the basophilic protoplasm that is the essential element of their life.

The elements of plant tissues have a similar constitution; microscopic study, supplemented by photographs, demonstrated that the elements of the parenchyma of a leaf, for example, have no cellular individuality and have the constitution just indicated; the constitution of plant tissues therefore differs notably from that currently accepted and described.

It can be said that ignorance of the nature and constitution of animal and plant organisms constituted the veil that made most biological phenomena impenetrable and mysterious. Indeed, the application of these new notions to the study of a certain number of them made it possible to achieve:

1) Knowledge of the nature of tuberculosis and cancer.

2) Knowledge of the spontaneous formation of bacterial cultures in the animal organism by transformation of specific organic mold and knowledge of a whole class of autogenous human diseases, bacterial and mycelial.

3) Knowledge of the nature of diseases that affect plants.

4) Knowledge of the original source of most pathogenic agents of human diseases.

5) Knowledge of the mechanism of phenomena such as specific hemolysis, the complement fixation reaction, and specific precipitation, attributed to the action of antibodies formed in the blood of animals.

6) Explanation of the phenomenon of anaphylaxis.

7) Explanation of the phenomenon of blood coagulation and its physiological purpose.

8) Knowledge of the nature of white blood cells in healthy animals.

9) Knowledge of the nature and composition of the ferments produced by plant and animal organisms.

10) Knowledge of the mechanism of immunity or vaccination.

A whole series of other knowledge results from the application of new concepts to the histological study of human tissues and organs and their physiological functioning. Let us briefly develop each of the listed topics.

1. Nature of Tuberculosis and Cancer.

Since human tuberculosis cultures were transformed into their gonidial forms of hyphomycetes, these were recognized as identical to the gonidial forms of human organic mold. Tuberculosis is therefore a spontaneous, autogenous disease of humans, transmissible by contamination, due to a pathological transformation of their specific organic mold.

Since the molds that constitute cancerous tissue were transformed by culture into their gonidial forms, these were recognized as identical to those of the specific organic mold of humans. Cancer is therefore an autogenous disease of humans, due to the morphological transformation of their specific organic mold.

2. Spontaneous formation of bacterial cultures in the animal organism.

The mold that constitutes animal and plant organisms can, like any mold, transform into bacterial elements; this transformation occurs easily in the organism of plants and animals. It is enough to freeze a potato for its organic mold to pass into the bacterial state; frostbite of animal tissue produces the same effect.

Inoculating an animal with the pulp of one of its tissues develops a bacterial culture; it also develops it more easily in the organism of another species; the culture always develops by inoculating, in broth, ground tissue.

Bacillus coli is a bacterial form of human organic mold. Many diseases, in which organs are invaded by Bacillus coli or other bacterial forms of organic mold, are autogenous diseases of the latter (liver and kidney diseases, cystitis, lung diseases, meningitis, etc.).

3. Nature of diseases that affect plants.

The parasitic agent of a plant disease is cultivated and transformed into its hyphomycete gonidial forms. The parenchyma of healthy plant tissues of the same species is cultivated to obtain the gonidial forms of the organic mold, which are recognized as identical to the gonidial forms of the agent known to be parasitic. The disease is therefore autogenous and due, like tuberculosis, cancer, and other human diseases, to a pathological transformation of the organic mold. It thus appears that plants do not become infected, or rarely do so.

4. Original source of pathogens of human and animal diseases.

Bacterial cultures of known pathogens were transformed into their hyphomycete gonidial forms. The still unknown pathogens of other diseases were isolated in their hyphomycete gonidial forms.

Comparison of these forms with those of organic molds of animals and plants made it possible to recognize in the latter the pathogens of human and animal diseases. Animals and plants, living or dead, are therefore, through their specific organic molds, the original sources of pathogens of animal diseases.

Thus, it was determined that the original source of viruses is the plant or animal indicated in the table below:

ORIGINAL SOURCE, Organic Mold from:

For Typhoid Fever, the organic mold of Corn.

For Typhus Exanthematosus, the organic mold of Oats.

For Paratyphus A, the organic mold of Wheat.

For Paratyphus B, the organic mold of Rye.

For Diphtheria, the organic mold of Barley.

For Malta Fever, the organic mold of Orange and Lemon.

For Epidemic Dysenteric, the organic mold of Brassica Species (Cabbage).

For Measles, the organic mold of Lettuce.

For Scarlet Fever, the organic mold of Carrot.

For Rabies, the organic mold of Carrot.

For Septic Vibrio, the organic mold of Carrot.

For Chickenpox, the organic mold of Tomato.

For Vaccinia, the organic mold of Potato.

For Smallpox, the organic mold of Potato.

For Foot-and-Mouth Disease, the organic mold of Potato.

For Tetanus, the organic mold of Potato.

For Syphilis, the organic mold of Monkey.

For Icterohemorrhagic Spirochetosis, the organic mold of Rat.

5. Mechanism of specific phenomena, such as specific hemolysis, specific precipitation, complement fixation reaction, attributed to the action of antibodies formed in the blood of animals.

All substances of animal or plant origin used as antigens contain the reproductive elements, spores and zymospores, of the organic mold of the species from which they originate. All tissues or organ extracts of animals and plants, their albuminoid substances, blood, serum, blood cells, egg albumin, etc., fresh or preserved in a dry state, contain the reproductive elements of the organic mold of the species.

These substances, injected into animals, develop a culture of the organic mold of the species from which they originate, and subsequently, the mycosis of this organic mold, along with the vegetative and fermentative elements (zymospores), develops in the animal the hyaline matter or mycofibrin, which forms the elements, similar to the fibrin in animal blood.

These vegetative and fermentative elements, and the hyaline matter of the blood of the injected or sensitized animal, transferred into an emulsion or solution of the antigen substance, determine the following phenomena:

The agglutination of blood cells is achieved by the hyaline matter, and their disorganization (or specific hemolysis) by the spores and zymospores of the culture, which clump together and draw them into the vegetative movement of the culture.

Specific precipitation, in the solution of a substance of animal or plant origin, is the formation of mycelial colonies caused by the elements provided by the precipitating serum.

Complement fixation is explained as follows: The active substance of complement, or alexin, is the acidic body that results from the hydrolysis of lecithins by a ferment (serozyme, which, when it comes to alexin, is called mittelstück); natural hemolysis is produced by this acidic body.

Specific hemolysis is produced by this same body and, certainly, when the red blood cells have fixed the sensitizer, which is their hyaline matter or mycofibrin formed in the sensitized serum, a matter eager for acidic bodies such as fibrinogen. Complement is said to be fixed when the acid body it releases is absorbed by a body other than the sensitized red blood cell.

The acid body is absorbed when the antigen bacterial culture and the sensitized serum containing the hyaline matter of this culture are added to the complement. There is coagulation of bacterial mycofibrin with absorption of the acid body.

There is therefore no difference between specific hemolysis, which is the fixation of complement by the red blood cell, and the fixation of complement by an antigen mixed with the sensitizing serum.

6. Mechanism of Anaphylaxis.

The state of anaphylaxis is the state of development of the mycosis of the organic mold of the animal or plant species that provided the antigen.

The proteolytic ferments, reported by Abderhalden in the blood of animals a few days after injection, are the zymospores of the culture, developed in the blood, of the organic mold of the animal from which the antigen emanates. They disappear from the blood when the antigen culture passes to the mycelial state, a transition which occurs, as in the most common human diseases, from about the twelfth to the fifteenth day after inoculation or injection of the antigen.

As is well known, the serum of anaphylactized animals produces a flocculation or precipitate in an antigen serum or antigen solution. This precipitate is known by the name anaphylatoxin; In reality, it is composed either of mycelial colonies or of clusters of spores in vegetation emitting mycelial filaments loaded with zymospores. This mycelial vegetation is determined in the antigen solution by the elements of the antigen mold (hyaline, spores, zymospores) brought by the precipitating, i.e., sensitized, serum.

Anaphylactic shock is due to the rapid agglutination of the elements of the antigenic mold caused by the injection of a certain quantity of the antigenic substance; in this agglutination phenomenon, the fermentative elements of the blood (spores and zymospores) are carried along, probably mechanically, with the elements of the antigenic mold.

7. Blood coagulation.

The formation of fibrin is the phenomenon of elaboration of the substance necessary for the mycelial development of any mold (hyaline substance). In animals, fibrin is the substance necessary for the mycelial development of organic mold.

Fibrinogen transforms into fibrin by binding the acidic portion of blood lecithins (cytozyme) hydrolyzed by the zymospores of the organic mold (serozyme).

8. Nature of White Blood Cells.

All molds develop by forming agglomerated masses or germinative masses. Normal white blood cells are agglomerated or germinative masses of the animal's specific organic mold. These are autogenous germinative masses.

In any disease caused by infection by a heterogeneous mold, the latter forms in the infected organism a considerable number of germinative masses which are leukocytes in the same way as autogenous leukocytes; but they are *heterogeneous leukocytes.*

The leukocyte index provided contradictory information because the leukocytes of the two species were combined in the same count. It has no meaning if the distinction is not made between the two species.

9. Nature and constitution of the ferments produced by plant and animal organisms.

These are the zymospores of their organic mold. These zymospores, very small granules driven by Brownian motion, are the active elements of the ferment. A ferment always contains, in addition to the zymospores, other larger elements, notably spores and germinative masses of the original organic mold.

The action of a ferment appears unlimited because the zymospores reproduce by germination of small agglomeration masses that they form or by germination of larger spores.

The action of a ferment cannot be proportional to the original mass because it multiplies.

10. Mechanism of Immunity.

Knowledge of the mechanism of immunity does not specifically result from knowledge of the constitution of the human organism, but this has made it possible to clarify, complete, and generalize the facts that explain the mechanism of vaccine action. These facts appeared successively during observations, without having been systematically sought with a view to explaining immunity.

The first to appear is the observation of the formation of leukocytes or germinal masses in in vitro bacterial cultures, and of identical leukocytes in the bodies of animals inoculated with these cultures.

Observation of how these leukocytes are formed, and confirmation of the existence of phagocytosis in vitro, demonstrated the misinterpretation that led to phagocytosis being called and, to the phenomenon of leukocyte formation being regarded as a defense phenomenon, which has precisely the opposite meaning; it is not a phenomenon of destruction of the pathogenic agent, but on the contrary one of the principal phenomena of its growth and development.

These observations led to the conclusion that the hitherto accepted existence of protective bodies or immunizing antibodies secreted by white blood cells, lysins, opsonines, etc., was impossible.

Observation of the mode of evolution of bacterial cultures in vitro and their evolution in the infected animal organism demonstrated that, in the latter, the acute phase of the disease corresponds to the bacterial development phase of the culture and that it is followed by a long-lasting chronic phase corresponding to the mycelial development phase of the pathogenic agent.

Inoculation of smallpox vaccine communicates the vaccine mold mycosis. The development of this mycosis, and of all mycosis in general, is accompanied by the development of agglutinating material (hyaline matter, mycofibrin) which causes the rapid passage, to the state of germinative masses, then to the mycelial state, of the bacterial elements which penetrate the organism in a new contamination.

In this way, the acute or bacterial phase of a disease is avoided or rendered insignificant; but this vaccination state *necessarily involves the infection of the organism by the pathogenic agent and the establishment of the mycelial phase of this infecion.* It is further demonstrated in this work that, like other diseases persisting in a chronic state in the organism in the form of long-term mycosis, vaccine mycosis or variola mycosis is the cause of late pathological manifestations (skin rashes, trichophytosis).

Knowledge of the constitution of the animal organism by an organized mold, which led to knowledge of the nature of the active elements in the organic products inoculated into animals (sera, albuminoid materials, blood cells, maceration of tissue extracts, etc.), made it possible to show that an identical mechanism governs the specific phenomena of hemolysis and precipitation, anaphylaxis, and the complement fixation reaction, to which capital importance was attributed in demonstrating the existence and formation by the organism of immunizing antibodies.

The mechanism of immunity is therefore very simple: it is the establishment of chronic mycosis or the mycelial phase of a disease that *confers immunity against the acute or bacterial phase only*. Immunity is therefore entirely relative and not absolute.

The protection of the mycelial phase against the acute phase is not a general phenomenon. The chronic mycosis of certain pathogenic molds does not confer this protection; this category includes diseases with repeated attacks: malaria, relapsing fever.

Finally, immunity cannot exist for diseases whose serious disorders are constituted by the mycelial phase: syphilis, tuberculosis, cancer.

Nor can it exist for autogenous diseases of the animal organism, since the mold that constitutes it vegetates in the mycelial state and, consequently, vaccination would confer the disease itself.

* * *

The aim of this exposition is, while providing a general understanding of the subjects studied in this work, to show how knowledge was acquired progressively, being checked and confirmed as it progressed naturally.

We will now move on to the systematic presentation of observations and demonstrations, following this same progression as much as possible.

* * *

RESEARCH STATEMENT

CHAPTER ONE

CONSTITUTION AND EVOLUTION OF BACTERIAL CULTURES

The determinism of the constitution and evolution of bacterial cultures is achieved by the study of the following points:

I. Bacteria have no individuality; they are only segmentation elements or spore forms of molds.

II. Determination of the elements of bacterial cultures.

III. Evolution of bacterial cultures in liquid media.

IV. Evolution of bacterial cultures on solid media.

V. Evolution of bacterial cultures in the animal organism.

VI. Return of bacterial cultures to the molds from which they came.

I. Bacteria have no individuality: they are only segmentation elements or spore forms of molds.

This proposition is demonstrated by the following facts:

1. A pure bacterial culture can be easily transformed into a mold. This fact is demonstrated at various points in this book.

2. In any culture of pathogenic mold, whether in liquid or solid media, we find the bacterial elements that characterize the pathogenic species (anthrax, Eberth, coli, syphilis, etc.).

Proof that these bacteria are indeed elements belonging to the mold can be given in the following way: spores are taken by lightly touching the surface of the mold with a wet stick, and then dipping it in a culture broth; a microscopic examination of the spores can be carried out beforehand to ensure that no other element has been taken.

The culture is heated in an incubator at 30° or 38° to obtain a bacterial culture; starting with the spores of a mold, this temperature of 30 to 38° is preferable, because a temperature of 15 to 20° is more favorable to pure mycelial growth.

Once the bacterial culture is developed, its microscopic examination will reveal the characteristic bacterial elements.

Certain molds, the *Aspergillus* form of organic potato mold, for example, transform quite easily, at 20-24°C, into a bacterial culture.

Certain aerial molds, even those obtained by transformation of bacterial cultures, then return with difficulty to the bacterial form when inoculated with spores from the gonidial apparatus. But often, over time, when a veil of aerial mold has developed on the surface of a liquid, the segmentation elements of the fine mycelial ramifications fall to the bottom of the culture vessel and constitute a deposit that contains the characteristic bacterial forms and will reproduce them.

3. Examination of the shape of the mycelial filaments and their partitioning, for a given mold, shows a perfect correspondence between the shape of the already segmented elements (bacterial culture) and the shape of the non-segmented elements, still constituted as mycelial filaments (diphtheria, glanders, syphilis, anthrax, etc.). When the bacilli come from the partitioned mycelial filaments, we find in certain cases, in the shape of these filaments, the reason for the characteristic shape of these bacilli; thus, the comma bacilli result from the segmentation of very wavy or spirally wound non-partitioned mycelial filaments (spirillae); depending on the length of the segmented fragments, the formed bacillus presents a single curvature or two S-shaped curvatures.

The transformation of a pure bacterial culture, by suitable processes explained later, reproduces the aerial pathogenic mold, the original source of the bacterial culture.

Multiplicity and characterization of bacterial forms of the same species.

The same species always possesses several bacterial forms; it can possess five or six different ones; it can possess at the same time and at the same time, in a culture broth, the micrococcus, coccobacillus, short bacillus, long bacillus, and spirochete forms, the latter being elements of the pathogenic agent in the same way as the bacilli, as will be demonstrated elsewhere.

The same species can possess, moreover, bacterial elements of different forms, succeeding one another in the cultures as they evolve.

The same species can also develop in a single bacterial form in the same culture and possess several different cultural forms.

Finally, these three cases can be combined in the same species; this is even very frequently the case. Here are the facts that explain this multiplicity of bacillary forms:

1. Bacterial cultures always come from a mold and are made up of the segmentation elements of its mycelium.

2. The molds (hyphomycetes) found in nature, growing on organic matter of plant or animal origin, always come from the organism of an animal or plant of which they are a constituent element.

3. The specific mold, which constitutes animal or plant organisms, always gives rise, by segmentation, to multiple bacterial forms, micrococci, bacilli of various forms, coccobacilli, spirilla, etc.

4. The organic mold of all plants or animals can give rise to spirilla; these form on the mycelial filaments of the gonidial forms *Mucor, Aspergillus, Monosterigmate, Sporothri - cum*, etc. The organic mold of all plants or animals possesses these gonidial forms.

5. The diversity of form of the bacillary elements comes from:

A. Because the mycelium of any mold produces branches of different types and sizes which, by segmenting, give rise to elements of various shapes.

B. Because any mold can develop in different gonidian forms (eight or ten for the same species, for example) and each of these has its own mycelial form which differs from the others; each of these mycelial forms gives rise, by segmentation, to distinct bacterial elements.

C. Because a bacillary culture undergoes, from the moment of its inoculation, an evolution that tends toward the regeneration of the aerial mold that gave rise to it. This evolution does not always take place toward the same gonidial form; it can result in different forms depending on the physical and chemical conditions that influence its development (temperature and nutrient medium conditions); the bacterial forms of the culture differ according to the gonidial form of the parent mold toward which it evolves; they also differ, on the other hand, according to the age of the culture and precisely because it evolves.

Throughout this work, we will find successively the demonstration of all the facts listed above, which it is not possible to develop here.

I will limit myself to citing a few examples:

Human organic mold gives rise to multiple bacterial forms: long and short basophilic bacilli, coccobacilli, very fine bacilli (Koch's bacilli), micrococci of various sizes etc.

Monkey organic mold gives rise to multiple bacterial forms, but it develops mainly in animal organisms *in the mycelial form*; its filaments give rise to a multitude of fine

spiral filaments which are *Treponema pallidum* or *Schaudinn's spirilla*; it is the agent of syphilis.

Organic molds of cabbage, lettuce, and carrot give rise to multiple bacterial forms; during the development of crops, various gonidian forms succeed one another.

Organic molds of tomatoes, eggplants, and potatoes give rise to multiple bacterial forms; but they can develop in crops in a bacterial form unique to a crop; thus, in addition to the common forms, there are two forms of cultures with a single bacterial type for organic mold of these plants: a culture containing only very large micrococci and a culture containing only large oval masses that multiply by budding.

All of these bacterial forms, as well as those of many other species, will be found in the study of pathogens of animal diseases. It is known that it is difficult to characterize a bacterial species using current methods and that this characterization can lead to errors.

The characteristic can be made with certainty by the transformation of the bacterial culture into a hyphomycete; this transformation is done fairly quickly and reliably by inoculating the culture into a guinea pig and culturing the pulp of its organs on agar; the comparison of the gonidian forms of the molds that develop in these cultures with the gonidian forms of the pathogenic agents makes it possible to know which of them corresponds to the culture inoculated into the guinea pig; it also makes it possible to know, for a good number of these agents, the original source of the culture, that is to say the plant or animal species that gave rise to it.

It is, for example, impossible or very difficult to identify a micrococcus by morphological characteristics or by those of cultures; but it will be very easy and safe to determine that it is *Micrococcus melitensis*, for example, when its *Penicillium* form has been obtained; it will easily be seen that it is that of the organic mold of oranges or lemons.

II. Determination of the Elements of Bacterial Cultures.

In initial observations, the various elements that exist in bacterial broth cultures were systematically examined and compared in cultures of various species: anthrax, tetanus, Eberth, coli, etc.

Later, I made these observations on bacterial cultures from transformed aerial molds, which are more suitable for such research because the evolution is more characteristic.

These observations showed that, in general, in a culture of a bacterial species, there is not only the inoculated bacillus, but various characteristic elements, each fulfilling a particular role in the culture. These elements are:

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1. The bacillary elements characteristic of the seeded species, at a given moment of evolution. At another time, one or more bacterial forms may have disappeared completely from the culture and been replaced by other *evolutionary elements*, that is to say by bacilli and cocci and by germinative masses formed by agglomeration of the latter.

2. Round hyaline granules of approximately 0µ.4 to 1 or 2 µm, barely or not stainable by basic colors; they are generally formed by endomycelial means or detached from hyaline sporiferous hyphae barely stainable by basic colors.

3. Granulations of 0µ.2 to 0µ.6, strongly basophilic; these are spores which, because of their fermentative role, will be rightly called *zymospores.*

4. Other granules of 0.2 to 0.5 µm which energetically fix basic colors and appear black in the preparations because they have fixed so much dye. I have not been able to determine if these granules are active elements of the cultures; it is likely that they are foreign to them, because similar ones have been observed in most liquids; however, they may be cholesterol granules, which fix basic dyes in the same way.

5. Basophilic granules with a diameter equal to the width of the bacillus: these are micrococci formed by its segmentation.

6. Agglomerated elements in varying numbers and in masses of different sizes. These masses are formed either solely from the hyaline granulations indicated above, or from these granulations to which bacilli and micrococci released by their segmentation have been joined. These are the germinative masses in the process of formation (Fig. 1, 2, pl. 1; fig. 2, pl. 6) in the culture.

7. In addition, there exists in the culture, either in dissolution or in a colloidal state so fine that the particles are not perceptible to the eye, a substance which coagulates and is deposited on the isolated elements. This substance can be seen around the elements and around the small agglomerations in plate 1, figure 2.

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This is the hyaline substance, the agglutinating substance which determines the adhesion and agglomeration of the elements. It is rare and difficult to see in some cultures. Others are rich in it. Its formation, probably due to the activity of zymospores, appears to depend on the chemical constitution of the culture medium and especially on its richness in nitrogenous matter.

It is this substance which, formed abundantly in the infected organism, constitutes *agglutinin*; its formation has no connection with a defense reaction of the organism; it is a product produced by the infecting agent and not an immunizing antibody.

These various elements exist in every culture. They are found in a tetanus culture made in the absence of air (Fig. 2, plate 2) as well as in a culture made in the presence of air. All these elements exist and can be examined under a magnifying glass in Figure 2, plate 6.

I add that, during evolution, the primitive bacillary form can be replaced by a different form; a second and even a third can also appear alongside the first (Fig. 1, syphilis; Fig. 2, tetanus, pl. 2).

If we examine at high magnification the culture placed between slide and coverslip with a little dahlia or methylene blue, we see:

1. That the zymospores, granules of 0.2 to 0.6 μm, are animated by a lively Brownian movement.

2. That they attach themselves to the hyaline or basophilic spores and to the bacilli and carry them along in their movements, giving them a double movement of oscillation and progression which should not be confused with an active movement; it is passive and caused by that of the granulations. It is the zymospores which operate the fermentative phenomena which take place in the culture broth; the other elements are the vegetative elements of the culture.

The nature and origin of the zymospores is certain and cannot be in doubt; in a culture or in a ferment solution, of which they constitute the active element, it is found that they are formed and carried, like the spores, by fine mycelial filaments developed by the germination of larger spores or germinative masses.

More detailed observations are provided on the properties of zymospores in the study of ferments and bacterial toxins. The study of toxins also includes the determination of the nature of filtering viruses.

III. Evolution of Bacterial Cultures in Liquid Media.

The phenomena of bacterial evolution were determined by microscopic observation of cultures of the first cancer-causing agent I isolated. Subsequently, I verified the observed facts by studying cultures of a large number of pathogenic agents and molds converted to the bacterial state.

To carry out these observations, I carried out broth cultures on glass slides. Five or six drops of glycosylated beef peptone broth are placed on a sterile glass slide, so as to form a prominent, circular liquid layer about twenty millimeters in diameter. This layer is seeded around its periphery **(1)** by needle punctures in five or six places, and ten or twelve similar slides are made for each species studied.

The slides are kept in a humid chamber, either in an incubator at a temperature of 38°C or at room temperature.

The cultures are examined every six or twelve hours or only daily. For the examination, the broth is evaporated on a hot plate, and the preparation is stained with dahlia and then washed.

These cultures showed me that the evolution phenomena are identical for all bacterial species. These phenomena are as follows:

1. From the moment of inoculation, there is agglomeration, into clusters of varying sizes, of the inoculated elements.

2. In a second stage, the elements fuse into the clusters. These clusters have a spherical or ovoid shape and a diameter varying from 5 to 30 or 40 µm, and even larger.

Often, the primitive clusters, the size of a leukocyte or even larger, come together and fuse, forming much larger masses. Identical clusters are found *in hyphomycete cultures and in human connective tissue.*

These agglomerated and fused masses are germinative masses; it is the sclerotia that will emit the mycelial filaments. These masses are very rich in basophilic protoplasm and take on an intense coloration when stained with dahlia or methylene blue.

3. In a third stage, basophilic mycelial filaments are emitted from the germinal masses. These filaments are continuous at the beginning of the culture's growth; no signs of compartmentalization can be discerned.

4. In a fourth stage, the mycelial tubes segment and give rise to new bacteria which, in turn, will undergo the cycle of the four transformations that I have just described: agglomeration, fusion, emission of mycelial filaments by the germinative masses, then segmentation of these.

These four stages, repeated, constitute the entire development of hyphomycete bacterial cultures until the determined moment when the evolution will stop definitively, the

(1) *Carefully, without stirring the liquid.*

most frequent case in bacterial cultures in broth, or will pass to the permanent mycelial state (transition to the hyphomycete state).

It is understood that one will not find exclusively in the culture, at different times, either free bacterial elements, or agglutinated bacterial elements, or germinal masses; the elements are mixed and the evolution continues identically *for groups of elements,* so that one always finds in the culture, at the beginning, segmented elements, germinal masses emitting mycelial filaments, and a certain number of these *in the process of segmentation.*

But the cycle of evolution is established by the existence of a large number of forms corresponding to all stages of this cycle. For example, one finds germinal masses in all stages of their formation; one finds some already emitting their mycelium and, alongside them, one sees long mycelial filaments in the process of segmentation, releasing bacilli identical to those already isolated in the culture.

Plate 1 shows these different forms of the elements in the four stages of the evolution of a mold obtained by transformation of a bacterial culture of tetanus **(1)**. We also note, by examining Figure 2 of this plate with a magnifying glass, that on most of the isolated elements, a substance is deposited that is not very stainable with dahlia and forms a cloud without a clear boundary around them. We also note that this same substance unites the agglomerated elements.

This substance is a sticky, adhesive material, the hyaline material; it is this which determines the adhesion or agglutination, and which constitutes *agglutinin.*

It develops normally in any bacterial culture, both in vitro and in the animal organism. In this same figure, we will find agglomeration or germinative masses at all stages of their formation, from the agglomeration of two elements to almost perfect agglomeration (2, 3, 4, 5, 6, 7). We will already note here the identity of these agglomeration masses with the leukocytes of animals. The germinative mass marked with the number 8 is an almost perfect leukocyte; it still lacks basophilic protoplasm which will be supplied to it by new bacilli which it will agglomerate as happens for mass no. 5.

The germinal masses of bacterial cultures do not differ from leukocytes. Normal leukocytes in the animal organism **(2)** *are born by the same process of formation as those in in vitro cultures and, like them, originate from the segmental and spore-bearing elements of a mold, the specific mold constituting the organism.*

(1) *Culture of a gonidian form (Hyphomycete) of tetanus, reduced to the bacterial state.*
(2) *Most leukocytes, but not all. Others have another origin, but are also mold elements.*

Animal leukocytes, like the germinal masses of bacterial cultures or molds, emit mycelial filaments called *protoplasmic extensions.* These extensions often bear, laterally, short-stalked spores or a terminal spore. They often bear mycelial dilations along their path. Details on this subject will be found at several points in this work.

As the culture evolves, it becomes enriched with elements.

Often, after a certain time, sometimes from the beginning of the culture, segmentation is not limited to the mycelium to form bacilli; the bacilli segment in turn until they form either short elements whose length does not exceed twice their diameter, or round elements, cocci.

As the culture progresses, the germinative masses often manage to emit both septate and non-septate mycelial filaments, similar to those of the aerial mycelial form of the hyphomycete. The sections of the septate filaments are often so slightly joined together that they are dislocated and separated into distinct elements by the slightest manipulation. As a result of this, two species of bacilli of different shapes are found in the culture.

For the same reason, three or four different bacillary forms may be found in a pure culture of a single species; this assembly of disparate forms, which is often found in infections, in certain abscesses, etc., has been called a "microbial association"; in this association there is generally only one and the same pathogenic species.

Very often, from the fourth day onwards or only on the eighth or fifteenth day, depending on the species, the germinative masses not only emit mycelial filaments, but also spore-bearing hyphae bearing round or oval spores; this fact can be observed, for example, by examining the deposit at the bottom of a tube of anaerobic tetanus culture.

Figure 2, Plate 2, shows the elements deposited in a one-month-old tetanus culture that Professor Legroux of the Pasteur Institute gave me. It shows:

1. Germinative masses emitting mycelial filaments.

2. Mycelial filaments of different types.

3. Segmentation elements (bacilli) of different types, other than hairpin bacilli

4. Mycelial filaments bearing fine lateral branches ending in a round spore.

These branches, equipped with their spores, detached from the mycelial filament that formed them, are the hairpin bacilli considered to be the agent of tetanus. They are in reality only one element of the segmentation of the tetanizing mold; the terminal spore, considered to be *the spore of the bacillus*, is only one of the multiplying elements of the mold.

Plate 3 shows elements of the development of a culture of tuberculosis in broth, either after seven days or in the light veil that it formed on the 28th day, and, by comparison, the elements of the same culture developed on agar after seven days. This is a culture obtained by inoculating in broth a portion of tubercular sputum collected in such a way as to avoid as much as possible contamination during emission. The culture, once developed, was transferred to agar. In Figure 1 of this plate, we see germinative masses (identical to mononuclear leukocytes) emitting their basophilic mycelial filaments, hyaline mycelial filaments **(1)** and fine, barely stainable segmentation elements which are distinguished mainly by the basophilic granules they contain (Koch's bacilli).

This culture slowly formed a veil on the surface. This veil, very fragile, was in the process of transitioning to a stable mycelial state. Placed on a slide, it became almost completely disorganized. Fig. 2 shows the elements: a portion of septate mycelium in the process of branching, and scattered elements originating from this mycelium. Figure 3 (agar culture) shows on the 7th day, in addition to scattered elements identical to those in the broth culture (Fig. 1), a group of agglomerated germ masses, each emitting (as in Figure 1) a mycelial filament which, here, is terminated by a spore - bearing hypha. Finally, Plate 239 shows perfectly, almost schematically, in Figures 1, 2, and 3, the cycle of evolution of the elements in a bacterial anthrax culture.

* * *

Summarizing these facts, we see that, in their evolution in liquid media, bacterial cultures pass through four successive stages: agglomeration, formation of germ masses by fusion, emission of mycelial filaments, and segmentation of these into bacterial elements. These four stages are renewed in successive cycles.

The shape of the bacterial elements varies with the age of the culture. Initially composed solely of elements of characteristic shapes in the first twelve or twenty-four hours, the culture may subsequently contain several other bacillary forms, spore-forming hyphae, or, after segmentation of the bacilli, contain only cocci. In general, the older it becomes, the more it contains agglomerated masses (some of them fused) of all kinds and very different sizes. These latter elements are called involution forms and are, in reality, normal evolutionary forms.

If the vegetation of the culture stops and if the germinative masses do not develop to a level that allows the transition to the perfect and permanent mycelial form, it is because the culture is grown in an environment that is too humid, at too high a temperature, or because it lacks oxygen.

(1) *A basophilic mycelial filament becomes a hyaline and poorly colored filament by flowing, through its end or its lateral branches, all of its basophilic protoplasm in the form of fine droplets (spores, zymospores).*

However, sometimes some germinative masses manage to do so and form large submerged mycelial colonies, which slowly end up invading the entire liquid, then, subsequently, forming a veil on the surface with the emission of aerial filaments.

The germinative masses can form immediately, and only on the surface of the liquid, a mycelial lascis constituting a non-villous veil. This veil can remain in this state or transition to the aerial hyphomycete form.

The two conditions that allow a bacterial culture in a liquid medium to transition to its aerial hyphomycete form are:

1) Culture at room temperature; a temperature of 30 is generally too high. The most favorable temperature is between 15 and 25.

2) Vegetation of the germinative masses on the surface of the liquid, that is, in contact with oxygen.

IV. Evolution of bacterial cultures on solid media.

In the study of cultures on solid media, agar, gelatin, carrot, potato, etc., we observe evolutionary phenomena identical to those I have just described. The culture shows, from the beginning, the passage of elements through the stages of agglomeration, fusion, germination of the agglomeration masses, then segmentation of the formed mycelium. As in broth cultures, we observe:

1. The diversity of bacterial forms for the same species.
2. The identity of the germinative masses with the leukocytes.
3. The formation of spore-forming hyphae.

In these cultures, the mycelial filaments emitted by the germinative masses have more consistency and can be more easily studied.

Plates 2 and 4 contain various drawings of elements constituting cultures on solid media. Plate 2 shows: Fig. 1, the elements of a syphilis culture on agar on the eleventh day; we see the multiplicity of bacillary forms, germinal masses of various shapes, and spore-bearing hyphae.

Figure 3, two branched mycelial branches and two spore-bearing hyphae in a gonococcus culture on the twenty-fifth day on agar.

Figure 4, various germinative masses and a long, branched mycelial filament in a forty-day culture of diphtheria bacillus. Two of these torn masses (on the left of the figure), whose contents are leaking, show that they were formed by condensation of hyaline matter having agglutinated zymospores.

This observation was made on the very culture that had been given to me. Later, I noted that the mycelium of the diphtheria mold was growing on the surface of this culture and that it bore characteristic fruiting bodies of *Citromyces*, which can be observed directly in the false diphtheria membrane dissociated on a slide and stained with dahlia.

Plate 4 shows:

1. The formation of germ masses in a culture of *Eberth's bacillus* on agar (Fig. 1). The culture is made on a thin layer of agar placed on a glass slide and kept in a humid chamber at 30°; the slide can be examined directly after weak dahlia staining. On the right of the figure, we see a group of germ masses and their first ramifications; on the left, an enlarged germ mass.

2. The emission of mycelium by the germ masses (Fig. 2). This figure shows two large, adjoining germ masses emitting multiple branched mycelial filaments. This is a broth culture of the guinea pig's organic mold (liver pulp culture) transferred to agar.

3. The stages in the development of a tetanus culture on gelatin in the first thirty-nine hours (Fig. 3).

After fifteen hours, strongly basophilic germinative masses the size of a white blood cell, or even larger, have already formed, through the fusion of hyaline elements and basophilic elements of varying sizes (0µ5 to 1µ5) numerous in the culture. There are also irregular, poorly stainable masses of hyaline protoplasm, having agglutinated basophilic zymospores visible in the masses, and which appear to be in the process of condensation.

After 23 hours, it is observed that these irregular hyaline masses emit long, very fine flexuous filaments which segment into very fine hyaline bacilli, poorly stainable, and visible above all by the more stainable granules they contain; these bacilli have an appearance identical to that of Koch's bacillus.

After thirty-nine hours, there are large germinative masses of various shapes emitting branched mycelial filaments. The observation of these various elements is difficult by culture on gelatin because it is necessary to melt it and dissolve it in water before coloring and, in this manipulation, the elements of the culture dislocate.

Bacterial cultures on agar, carrot or potato, frequently transform into the state of hairy aerial mold (hyphomycete). This transformation, which all bacteriologists must have seen, was considered by them to be due to contamination; it is normal, and mold is

indeed an evolving form of bacterial culture **(1)**. It occurs most easily at the top of the culture tubes, at the point where the agar, having the least thick, dries out most easily, and it is often when this desiccation reaches a certain degree that the transformation takes place. After observing this fact, I used it systematically to obtain the transformation of bacterial cultures into hyphomycetes.

V. Evolution of Bacterial Cultures in the Animal Organism.

Transported into the animal organism, the bacterial culture of a pathogenic agent evolves as in an in vitro culture medium. Studying this evolution in one or the other of the two environments consists of observing the formation of germinative masses, their germination, and the segmentation of the mycelium.

This simultaneous study of the culture of a pathogenic agent in the animal organism and in an artificial in vitro medium is very interesting because in vitro culture allows control of the forms observed in the animal organism. Let us cite a first example: A tetanus culture is made on gelatin as indicated previously. The elements developed in such a culture have already been described previously (Pl. 4, fig. 3).

On the other hand, two or three drops of a tetanus culture are introduced into the rat's pleura. The animal dies of tetanus within twenty-six hours. A drop of pleural fluid (which does not contain blood cells) is withdrawn, spread on a slide, and stained with dahlia. The elements it contains are shown in Plate V. It shows germinal masses (leukocytes) in the process of forming (1, 2, 5, 6, 7, 8, plate 5), germinal masses that have already emitted one or more mycelial tubes (9, 10, 11, 12, 13), germinal masses whose mycelial filament is a spore-bearing hypha (4), and finally germinal masses (3) (sclerotium) emitting a very developed mycelial network.

Here, the germinative masses are formed by the union of a few basophilic granules around which a mass of hyaline matter condenses. The large basophilic granules, which will constitute the germinative mass by their agglomeration, probably have several origins. The largest come from the sporiferous hyphae (4). These same granules of the same size are found in the gelatin culture. Certain masses (5, 6, 7, 8) are formed by progressive agglomeration; they take on the appearance of mononuclear and polymorphonuclear leukocytes. It is the contribution of basophilic granules to these masses which constitutes their nucleus: we can clearly see that these granules, initially simply attached to the masses, penetrate into their interior and fuse there.

(1) *Which is not, however, purely bacterial, because it most often contains evolving and highly developed mycelial elements bearing hyphomycete fruiting bodies.*

This shows that polymorphonuclear and mononuclear leukocytes are only successive stages in the formation of germinative masses. In fact, the normal leukocytes of the animal organism are, like those in question here, germinative masses of molds.

Other masses (1, 2) appear to acquire their definitive size immediately by agglomeration of basophilic spores identical to those of the spore-forming hyphae, around a small, already fused mass which constitutes their central nucleus (1). Masses of this kind are observed in a tetanus culture on gelatin from the fifteenth hour.

These various germinative masses emit mycelial filaments. Plate 5 represents one of these masses (2), similar to masses 1, 2 and the highly branched filaments it emits. Other germinated masses, 9, 10, 12, 13, still contain a nucleus and granulations like masses 5, 6, 7; if they are no longer as vividly stained by the dahlia, it is because their basophilic protoplasm has partly emerged in the form of mycelial filaments and especially in the form of basophilic spores detached from the mycelium.

It could be argued that, in this experiment, germinal masses 5, 6, 7, and 8 do not surely belong to tetanus and come from the rat's organism. Comparing germinal masses in a gelatin culture and even in broth allows us to answer this objection.

Another example will provide us with even clearer demonstrations of the mode of formation of germinal masses in molds and the origin of leukocytes in the animal organism.

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I collected from unsweetened black coffee, left in a flask, a mold that had grown spontaneously there. This coffee gave off an infectious odor; replanted several times, this mold was determined to be a pure culture of a *Penicilium* **(1)**; it causes an infectious fermentation in the culture broths on which it develops as a veil of hyphomycete. The broth deposits bacterial elements at the bottom of the vessel. These elements, transferred to the broth, develop exclusively in bacterial culture, and give it the same infectious odor.

Examining this culture, which is the bacterial form of a perfect hyphomycete, a *Penicilium*, we see it constituted by five or six diverse forms of bacilli, by several species of spores, by zymospores and finally by germinative masses. If we examine a twenty-four-hour culture, we find it to be made up of a single bacillary form: bacilli of various lengths segmenting; we also find germinative masses in formation.

(1) *According to the rule I have observed here, I will not give a specific designation of this Penicilium, because it is established elsewhere that the various types of Penicilium are not species and that ten or twenty types, appearing exactly similar, which we will designate, for example, Penicilium candidum, may belong to ten or twenty different species.*

I photographed one of these germinative masses, very clear, isolated, surrounded by bacilli (Fig. 2, pl. 6. Magnification 2,200 by enlargement: 1,100 × 2). This mass is constituted by hyaline granules (spores) and by basophilic granules (micrococci formed by segmentation of the bacilli). It is a typical leukocyte in the process of formation. In addition, we notice that two whole bacilli have agglomerated in the mass; they will contribute to forming the nucleus.

This phenomenon of agglomeration and fusion of bacilli was already visible in Plate 1 (Fig. 2, item no. 5), a photograph of another hyphomycete bacterial culture.

This is the phenomenon of phagocytosis, which occurs both in an in vitro culture and in the animal organism.

Furthermore, I injected a drop of the bacterial culture into the cerebrospinal fluid of a dog. If this cerebrospinal fluid is examined after 24 hours, it is found to be cloudy and contain an enormous quantity of leukocytes. Figures 1 and 2, Plate 7, are photographs of a preparation of this fluid, stained with dahlia. Figure 1, Plate 6, is an enlarged photograph (Gr. 2.200) of Figure 1, Plate 7.

We see all the stages of the development of a leukocyte from the beginning of agglomeration to the final state of germinative mass, a state which is that of the leukocyte whose nucleus, colored intense blue by the dahlia, occupies the entire mass. By examining a leukocyte in detail under a magnifying glass, we find it constituted exactly like the previous leukocyte in the in vitro culture. We see, as in this one, the contribution of the same bacillus inoculated to constitute the nucleus.

This contribution of bacilli is proof that the leukocyte indeed belongs to the injected pathogenic species, agglomeration taking place only between elements of the same species. This conclusion is demonstrated to be correct by the specificity of the agglutinating action.

The presence of leukocytes, or germ masses, is constant in bacterial cultures and also in hyphomycete cultures. Germ masses do not always have the origin and mode of formation just described. I cite an example:

I injected into the cerebrospinal fluid of a dog spores of a mold growing spontaneously on bread and which presented the morphological characteristics of a *Mucor racemosus*. After twenty-four hours, the fluid contained a considerable number of spherical bodies of 4 to 6 v. which I took for lymphocytes and large germinal masses of 9 to 14 μ. By examining preparations obtained by rapid desiccation of the cerebrospinal fluid and staining with dahlia, I saw that the free lymphocytes in the fluid were balls detached from the end of mycelial filaments born on the germinal masses of 9 to 14 μ. (Fig. 1, 2,

3, pl. 8). The latter also emit fine, highly branched filaments bearing a multitude of small appendages, straight or spiral, bearing at their ends very fine spores.

These facts demonstrate that, in an infected organism, we find lymphocytes or leukocytes from several sources in the blood and other organic fluids:

1. Those from the normal organism.
2. Those from the current infection.
3. Perhaps those from previous persistent infections that have become chronic.

The nature of leukocytes, thus established by observing the development of bacterial cultures in vitro and in the animal organism, was subsequently corroborated by the demonstration of the nature and origin of animal and plant tissues.

I will develop this subject more fully elsewhere, by presenting new observations that provide other elements of appreciation.

* * *

Let us recapitulate the facts established so far by the preceding observations and draw the conclusions they entail:

1. Bacteria have no individuality and do not reproduce individually by spores or fission. They are the elements of the segmentation of the mold mycelium or its fruiting bodies (spores).

2. Molds are the pathogenic agents of infectious diseases.

3. In every bacterial culture, there is the formation of a considerable quantity of zymospores, very small granules (0.2 µm to 0.5 µm) driven by Brownian motion and possessing fermentative power; due to this movement, they pass through porcelain filters. *They constitute filtering viruses. These do not actually exist, as zymospores constitute only the smallest elements of pathogenic molds*, *the larger ones that accompany them having always escaped the attention of observers.*

4. In their development in culture immersed in a nutrient liquid, molds give rise to a bacterial culture; their elements evolve by passing through four successive stages: agglomeration of isolated elements, fusion of these elements to form germinative masses, germination and formation of mycelial filaments, segmentation of the mycelium into bacterial elements, and direct formation of external spores on the mycelium.

5. The formation of germinative masses is determined by the coagulability of a hyaline substance that is deposited on the isolated elements and causes their agglutination and agglomeration. This substance also forms, by condensation, protoplasmic masses to which isolated hyaline and basophilic elements are joined.

6. Leukocytes are formations of the development of molds. They exist both in an in vitro mold culture and in the animal organism.

7. In an infected organism, a portion of the lymphocytes or leukocytes is made up of germinative masses of the infecting mold, which should not be confused with the body's normal leukocytes.

8. *Phagocytosis is not a phenomenon of the body's defense; This is a normal phenomenon in the development of any mold: the agglomeration of bacterial elements into germinal masses, with a view to their formation.*

9. The disappearance of bacteria from the humors of an infected organism does not mean that they have been destroyed by a lysine; it only means that they have evolved and changed form by passing into the state of germinal masses. This disappearance therefore does not mean that the disease is cured; it only means that it is moving to another phase (chronic phase) and that the acute or bacterial phase is over.

10. The nature and origin of leukocytes, the non-existence of phagocytosis as a defense process, the disappearance of bacteria solely due to the natural evolution of pathogenic molds constitute the demonstration:

A. Of the non-existence of lysines and opsonines, immunizing antibodies which would be created by the body's defense reaction.

B. On the lack of significance of the leukocyte index as established so far. It could only have significance if a distinction were established in the count between the normal leukocytes of the organism and those of the pathogenic agent.

Having determined that bacteria are only the products of the segmentation of the mycelium of molds, this being one of their means of multiplication, we will now study:

1. The means of returning a bacterial culture in vitro to the aerial mold from which it originates.

2. The fate of leukocytes or germinal masses of pathogenic molds in the animal organism, after the disappearance, at least apparent, of the pathogenic bacterial elements in the humors.

VI. Return of bacterial cultures to the aerial molds from which they originated.

The fact that bacteria have no individuality and are only segmentation elements or spores of molds, implied that it should be possible to return, to the hyphomycete form

that gave rise to them, all the pathogenic bacterial cultures currently preserved and reproduced in bacteriology laboratories.

Bacterial cultures are preserved and perpetuated in the same form because they are cultivated at 38°C and in liquid media or on solid media whose constitution is not favorable to mycelial development.

However, it is not uncommon for these cultures, especially when they are old, to transform into the original aerial mold; in this case, bacteriologists consider the mold that appears to be a defilement.

At first, I succeeded, but with difficulty, in obtaining the transformation of a certain number of bacterial cultures on various media: agar, carrot, potato, meat, coagulated casein, etc.

Various observations showed me that the most favorable conditions for the transformation of bacterial cultures into aerial molds are:

1. Culture at a temperature of 15 to 25°C (optimum 20 to 25°C).

2. Culture on a solid medium that is not too humid

3. A culture medium rich in sugar and albuminoid matter.

The media that have given the best results are agar, coagulated casein, cooked beef, and cooked egg yolk. In cases where these media have not been satisfactory, I have obtained a positive result by placing the bacterial culture on a piece of thick refractory earth soaked in broth placed in a thin layer at the bottom of a sterile flask.

The transformation sometimes occurs in one week, sometimes in two, three, or four weeks, sometimes after only several months.

To obtain it more easily, it is necessary to inoculate the deposit that exists at the bottom of broth cultures, or better still, the bacterial culture on agar.

It is advisable to spread the culture in a thin layer in certain places and, in others, to arrange it in small piles.

By these means, I succeeded in transforming most pathogenic bacterial cultures into one or more gonidial forms of the mold from which they came.

The first transformation I obtained was that of *Eberth's* bacillus. I could not doubt the accuracy of the result since, after several subcultures of the mold, *Eberth's* bacillus was always found in the middle of the mycelial filaments and on the surface of the culture medium.

I then obtained the transformation of cultures of coli, anthrax, cholera, tetanus, Koch's bacillus, diphtheria bacillus, staphylococci, streptococci, etc.

The transformation attempts, so demonstrative at first, were stopped by a difficulty that at first seemed insurmountable. In the transformation of a culture of anthrax, I obtained two molds, a *Penicilium* and an *Aspergillus*. By transforming two cultures of tuberculosis, I obtained, from one, a *Penicilium* and a *Sporothricum*, from the other, a *Penicilium* and an *Aspergillus*.

I had to conclude from these facts, by applying current mycological knowledge, that the molds obtained were different species originating from elements foreign to the pathogenic germ and either contained in the original cultures or introduced by my manipulations.

New tests, carried out while carefully avoiding any cause of contamination and with new cultures, the purity of which was guaranteed to me, gave the same results.

These results being inexplicable and appearing incoherent, I sought to obtain the transformation of the cultures by another process. The previous method is, moreover, very long, and it was desirable to obtain a faster one.

I had noticed that, on the one hand, transformation is all the easier the richer the inoculated culture is in germinative masses or the more easily it forms them, and that, on the other hand, the molds injected into the animal organism quickly form a considerable number of these large masses.

These facts led me to try injecting the bacterial cultures into the animals, reducing their organs, enlarged lymph nodes, liver, spleen, lungs, to a pulp, and culturing this pulp on sugar agar. The result of this test was conclusive because, *always,* the pathogenic mold appears from the third to the fifth day on the organs thus cultivated.

The comparison of the forms of hyphomycetes thus obtained, with those that the direct transformation of the bacterial cultures had already given, left no doubt about the accu-acy of the process.

However, this method presented a serious source of error. Indeed, alongside the pathogenic molds, I obtained molds that had nothing in common with them; there seemed to be several of them, because I obtained them under varied types: *Penicilium, Aspergillus, Sterigmatocystis, Monilia*, etc.

This fact appeared to me as proof either of contamination existing in the bacterial cultures that I had from the Pasteur Institute and the Faculty of Medicine, and already

suspected in the process of direct transformation, or of the normal existence of parasitic molds in animals.

I consequently made cultures on agar of the pulp of the organs of normal guinea pigs and rabbits; molds did indeed develop there and, astonishingly, I noted that these molds *were always the same for the guinea pig, different in the rabbit, but always similar in it*; I found the same result for the rat and the mouse. This fact therefore proved that part of the foreign molds in the injected culture came from the organism of the animals.

I was able to avoid this second cause of error by simultaneously inoculating the guinea pig and the rabbit, whose normal molds are different, and by eliminating these, through careful control, from the molds obtained from the organs of the inoculated animals.

It is therefore necessary, for this control, to have isolated and studied the species of mold that usually develop in cultures of the pulp of the organs of the guinea pig and the rabbit.

I later observed, through experience with a large number of cultures, that the molds that develop from the third to the sixth day on the pulp of organs almost always belong to the inoculated pathogenic agent and that confusion with the normal molds of the guinea pig and rabbit can rarely occur. It is especially after the sixth or seventh day that these latter molds develop. Subsequently, the explanation of their origin and nature was given by demonstrating the nature of the constituent elements of the animal organism.

At this time, the safety of the procedure was further increased by this knowledge; with experience, it became rigorously reliable.

As soon as I was assured of the safety of the process of rapid transformation of bacterial cultures by passage in guinea pigs, I checked the results of my direct transformations on solid media and found them to be accurate. But, as with the use of the direct transformation process, I observed that several types of mold were growing alongside the pathogenic mold, which was surely the real form since it was similar to the direct transformation form.

For tuberculosis and anthrax, I found exactly the same molds already obtained by direct transformation. There was therefore a certain fact, not accidental, which had to be explained.

I investigated whether there was a relationship between these various types obtained with the same pathogenic mold. I separately inoculated several guinea pigs with the

different types of molds obtained, and I compared the various forms that vegetated on cultures of the pulp of their organs. The result was rigorously demonstrative.

By inoculating the guinea pig with the pure *Aspergillus* form of anthrax, I obtained the *Penicilium* form already obtained directly. By inoculating the *Sporothricum* and *Aspergillus* forms of *Koch's bacillus*, I obtained the same *Penicilium* form already obtained directly as well. The repetition of these tests on other molds rigorously confirmed these results, the significance of which was:

1. That the same species of mold occurs in many different gonidial types: *Penicilium, Aspergillus, Sporothricum, Mucor*, etc.

2. That these gonidial types are not genera as they have been considered up to now, but only fruiting or vegetative forms that can all belong to each mold.

These facts were the origin of the research set out in the chapter *"Multiplicity of Gonidial Forms of the Same Species"*; I indicate here, so that there is no doubt, that the obtaining of proofs of this multiplicity was so complete that I observed *the direct birth of the fruiting mycelial filaments of the Penicilium form on the mycelial filaments of the Mucor form.*

From these demonstrations, we must retain:

1. That the transformation of a bacterial culture into a hyphomycete provides decisive proof of the origin of bacterial elements and the *nature of the pathogenic agents of contagious diseases: they are molds*.

2. That animal tissues and organs always, normally, contain mold elements.

Knowledge of the means of culturing pathogenic agents, either in bacterial form or in mold form, and especially knowledge of the culture process by passage through the guinea pig's organism, allowed me to isolate the pathogenic agents of most diseases with unknown germs: *measles, scarlet fever, chickenpox, vaccinia, smallpox, typhus, foot-and-mouth disease, lethargic encephalitis, rabies, etc.*

I was also able to acquire new knowledge about the pathogens of other diseases, such as syphilis, sleeping sickness, and malaria.

The isolation and culture of the syphilitic mold, which I carried out at this time, showed that T*reponema pallidum* is not a protozoan, but a simple spiral filament arising on the mycelium of the mold; a large number of molds of the types *Mucor, Aspergillus, Sporothricum*, and *Monosterigmate*, carry on their mycelial filaments a multitude of these small spiral filaments which, when detached, constitute the elements that have been called spirillae or spirochetes.

The isolation and study of the mold that causes malaria showed that Laveran's hematozoa is a spore or evolving germ mass of this mold. This agent therefore has nothing in common with a hematozoa.

The isolation of the mold that causes sleeping sickness showed that trypanosomes are not hematozoa, but mycelial formations of the pathogenic mold.

* * * * *

CHAPTER II

EVOLUTION OF PARASITIC MOLDS IN THE ANIMAL ORGANISM IN THE MYCELIAL STATE

In this chapter, we will successively study:

1. The fate of heterogeneous leukocytes in the animal organism and the establishment of mycelial vegetation.

2. The forms taken in the animal organism by the molds that vegetate there in the mycelial state.

* * *

I. Fate of heterogeneous leukocytes or germinative masses of pathogenic molds in the animal organism after the disappearance, at least apparent, of the pathogenic bacterial elements.

Establishment of stable or permanent mycelial vegetation of pathogenic agents in the organism.

We have just seen that a bacterial culture or a pathogenic mold, introduced into the body, forms:

1. Bacterial elements, bacilli or micrococci.
2. Spores.
3. Zymospores, agents of bacterial fermentation.
4. Germinal masses.
5. A probably dissolved, coagulable or precipitable substance, hyaline matter, the agent of agglomeration or agglutination.

It has just been demonstrated that the germinal masses of the infecting agent are leukocytes.

It is probable that the increase in the number of leukocytes in the blood or humors, observed in infections, is due solely to the heterogeneous leukocytes formed by the infecting agent.

Proof of the existence of these germinative masses in the blood of infected individuals is easy to see; the blood of patients with measles, scarlet fever, malaria, etc., contains a

large number of them alongside the other elements of the infecting agent: bacterial elements, spores, mycelium; it even contains the normal fruiting bodies of the infecting mold. Evidence of the formation of these germinative masses in the body has been given previously.

The disappearance of pathogenic bacteria from the blood of a patient corresponds, not to their destruction, but to their transition to the state of germinative masses.

We will now examine the fate of the elements of the culture in the body.

It has been shown that phagocytosis is not a defense phenomenon; it is a normal phenomenon in the evolution of molds; the bacteria agglomerate to form germinative masses which are leukocytes.

If bacteria disappear from the blood of a cured patient, this does not mean that they have been destroyed; it only means that they have changed form, as in an old culture broth. In a culture broth, these transformation elements are easily found; in the blood, they were not seen because the heterogeneous leukocytes, formed by the infecting agent, were confused with normal blood elements that have a roughly identical shape.

The disappearance of bacteria from the blood and their total or almost total transformation into germinative masses *is therefore a phenomenon of evolution of the infecting agent and not a phenomenon of defense;* it is caused by the development, in the organism, of the hyaline matter of the infecting mold, a substance which gives the serum the *specific agglutinating power,* that is to say, limited to the infecting bacterial species.

Germination of agglomeration masses or heterogeneous leukocytes.

The agglomeration masses or heterogeneous leukocytes, once formed, emit mycelial filaments as they do in cultures on artificial media.

This has been demonstrated for tetanus cultures, either on gelatin or in the organism (rat pleura).

The fact is easily demonstrated. It is enough to examine the blood of patients suffering from scarlet fever or measles to find germinative masses emitting oxycelial filaments. In scarlet fever, they emit very fine filaments, very long, tangled in balls. To see them, they must be looked for in the blood made incoagulable by sodium citrate, or in the clot that surrounds them.

Plate 9 shows an enormous germinative mass **(1)** of blood (obtained by pricking the ear) from a guinea pig inoculated with *Trypanosoma gambiense*, made available to me

by Professor A. Petit, of the Pasteur Institute, whom I thank for the various study elements he was kind enough to provide me.

This germinative mass emits a group of mycelial filaments terminated at their tips by *Trypanosoma gambiense*. These are therefore not protozoa, but dilations detached from the tips of the mycelial filaments of the mold that causes sleeping sickness. Figure 2, Plate 10, shows another germinative mass 1 contained in the blood of a guinea pig inoculated with one of the gonidial forms of the mold that causes the disease. This mass emits mycelial filaments, one of which, 3, is a *Trypanosoma gambiense.* In Plate 10 we see:

1. A germinative mass in the process of germinating in a broth culture of anthrax(Fig.1).

2. Germinal masses emitting their mycelium into the cerebrospinal fluid of a guinea pig inoculated with one of the gonidial forms of the tetanus mold (Fig. 3).

3. Germinal masses contained in the sputum of pneumonia patients and the mycelial filaments they emit (Fig. 4). These filaments often bear dilations at their ends which, if detached, could be mistaken for trypanosomes.

4. Germinal masses growing in breast cancer. Often, upon their emission, the mycelial filaments bear spindle-shaped dilations (3, 4, Fig. 5). One of the masses (2, Fig. 6) emits the fine branched mycelial filaments that have been called the pseudopodia of the white blood cells.

These filaments generally give rise to a large number of zymospores carried on a very short pedicle **(2).**

The formation of the mycelium remaining adherent to the germinative masses or heterogeneous leukocytes makes their further destiny evident. The whole mass and its mycelium, having become too large, can no longer cross the capillaries and is stopped at some point in the tissues where it will continue its vegetation and will determine the pathological phenomena of chronic diseases (diseases of the liver, kidney, lung, nerve centers, etc.).

(1) *The periphery of the mass is masked by attached blood globules. The black granules scattered in the field are globules. Staining by the Laveran method is poorly successful.*
(2) *This origin is not visible in the figure because, like Figure 6, it is a drawing, and, believing I was faithfully copying the observed image, I did not see, at the time I executed it, the position of the zymospores on the filaments; such facts have shown me that drawings are always inaccurate and that it is strictly indispensable to photograph the observed elements. Photographs, which represent rigorous accuracy, are even more favorable for study than direct examination by the eye.*

Disorders that occur remotely and consecutively with certain diseases, such as diphtheria, cerebrospinal meningitis, typhoid fever, scarlet fever (disorders called sequelae of various diseases, etc.), have been attributed to bacterial toxins altering brain matter and other tissues with which they are combined. It has been shown that these toxins are formed elements of an appreciable diameter and not soluble elements capable of such chemical combinations. This fact alone would make the explanation accepted until now improbable. The persistence of the pathogenic agent in the tissues, in mycelial form, provides a direct explanation for the observed disorders. Any heterogeneous mold lives in the animal organism at the expense of the tissue elements in which it vegetates and which it destroys, like the carrot or potato on which it is cultivated in vitro.

The reproduction of pathogenic mold, by culturing the pulp of tissues from animals inoculated for several months, demonstrates the existence of pathogenic molds in a stable mycelial state in the tissues.

If another demonstration were necessary, it would be found in the persistence of malaria mold for a good number of years in humans and by the permanent persistence of syphilitic mold.

All the facts established so far prove:

1. That the bacterial species that have been considered until now as the pathogenic agents of diseases are only the segmentation products of pathogenic molds, the true agents of diseases.

2. That the acute period, bacterial or fermentative, of diseases is only the first phase of the pathogenic action of heterogeneous molds.

3. That after the acute phase of diseases has ended, pathogenic molds continue to vegetate in the tissues in the mycelial form, and are the cause of the chronic period that follows the acute phase (mycelial phase).

The reappearance of bacterial elements in the blood of a malaria patient at the time of an attack shows that, in certain diseases, the mycelial phase can involve frequent, even periodic, returns to the fermentative phase.

Permanent or long-lasting mycoses.

Pathogenic molds therefore cause, after the acute period or apparent cure of diseases has ended, mycoses of a certain duration or are permanent.

When these observations were made, I looked for traces of these mycoses in the organism, that is, to detect the vegetation of the molds that cause them. This research

demonstrated the existence, in all animal tissues or organs, of vegetative elements of molds; it demonstrated at the same time that these elements, as well as their fruiting bodies, take special forms in the organism that often make them difficult to recognize.

This observation required an additional study on the vegetative and fruiting forms of molds in vitro with a view to identifying them with those that I had observed in animal tissues.

In possession of this new element of appreciation, I was able to undertake a new study with a view to systematically determining the elements of parasitic molds and their relationships with the normal constituent elements in each organ.

In the following studies, we will therefore examine in succession:

1. The vegetative and fruiting forms of molds in the animal organism.

2. The identification of the vegetative forms observed in the animal organism with those of molds cultivated in vitro.

3. The study of the normal constituent elements of tissues and their relation- ships with the elements of parasitic molds.

II. Study of the vegetative forms and fruiting bodies of molds in the animal organism.

The search for these vegetative forms was carried out in all the tissues and organs of animals: connective tissue, liver, spleen, lung, nerve centers, etc. It was carried out on preparations obtained by scraping the section of the organ with the blade of a scalpel, depositing a little of the pulp obtained in a drop of water, mixing, then spreading it on a glass slide, rapid drying by heat, staining with dahlia and washing. For the connective elements only, the preparations were made by dissociation and spreading, dry, on a slide (connective tissue, choroid plexuses, etc.).

When this research began, I was poorly equipped and unfamiliar with microscopic photography of preparations. I therefore initially recorded my observations on drawings. I was, however, able to obtain photographs of my preparations fairly quickly to record a certain number of observations using this process, which, from that moment on, was the only one used.

In the course of this study, it was the rat and the mouse that presented the most varied vegetative or fruiting forms and that seemed to me to be the animals of choice for observation. Therefore, it is with them that the description will begin.

We will first examine the most common fruiting forms of the grey mouse.

Grey mouse. We observe:

1. a type of fructification in the form of a spherical ball (1, pl. 11), which is, most often, the primitive form of development of the spore-bearing ends of the *Citromyces* or *Penicilium* forms.

2. A type of terminal ball-shaped fruiting bodies, either regular (6) or irregular (7, 8), formed of granulations which are not very colourable by dahlia (7, 8) or, on the contrary, which fix this colour quite well (6). These balls are found, in a very similar aspect, in the first stages of the development of certain *Penicilium* or *Citromyces* whose fruiting bodies develop in spherical or pear-shaped protoplasmic masses.

3. A type of fructification in spindles (2, 3, 4, 9, 10, 12, 14, 16, pl. 11); these correspond to the end of a branched gonidiophore bearing several groups of sterigmates remaining attached. This type corresponds to the cylindrical or spindle shape of the fructifications of *Epidermophyton, Achorion gypseum,* and the spindle organs of *Trychophyton* and *Microsporum.* It is demonstrated elsewhere that these molds easily pass to the *Penicilium* type and that, in this case, the groups of sterigmates of the latter develop in the spindles. Some spindles are curved into a crook and have, at the convexity, projections, each corresponding to the end of a group of sterigmates in formation. This type is frequently found in cultures of certain *Penicilium* at the beginning of their development.

These different spindle formations often have the characteristic appearance of trypanosomes (2, 4, 14, 16). I believe that the trypanosomiasis that has been described in rats has this origin. It has already been previously indicated that trypanosomes are dilations of the end of mycelial filaments of molds. The research that follows in this work shows that rat trypanosomiasis must be constituted by the release, into the blood, of *normal* formations probably detached from the spleen.

4. A type of pear-shaped fruiting body (13,5), in which the different groups of sterigmates are often marked by a projection and a groove separating them. This type is also found in in vitro cultures of *Penicilium*.

Other fruiting bodies of very varied types are also found, all of which would take too long to describe.

For example, we observe:
Spindle-shaped mycelial dilations, ending in a clover-shaped fruiting body (11);

Spindle-shaped dilations, often voluminous, whose tapered end bears a ball-shaped fruiting body (17, 18);

Leaf-shaped mycelial dilations.

Plate 13 contains four photographs of a preparation of the mouse spleen. Examining them under a magnifying glass, one will find most of the forms gathered in Plate 11.

All the formations I have just described are made up of fine branches of the formative shoot, highly ramified, tangled, and bearing hyaline spores of various sizes.

Rat. The same types of fructifications are observed in the spleen of the rat (Fig. 3, pl. 15, from a drawing).

Plate 13 contains four photographs of preparations of the spleen of this animal. Examining them under a magnifying glass, one will find most of the forms just described.

Figure 4 of this plate, made at a high magnification (1100 D), demonstrates that the fruiting formations are grouped on branched filaments, and that the mycelial filaments which carry them are not, as a histologist might judge at first glance, artificial protoplasmic trails, artefacts. The same fact is observed in the mouse (4, 5, pl. 11).

Moreover, to establish his conviction, the observer has only to follow the path of a sufficient number of mycelial filaments. He will find some which carry at their end a hyaline spore, others which carry small mycelial dilations spaced along their path, like the dendrites of the cells of the cerebral cortex, and others finally which clearly show the characteristics of the mycelial filaments. On preparations made by placing on a slide the product of scraping with a scalpel the fibrous framework of the spleen, exposed by prior persistent scraping, we will observe the presence of large mycelial branches, easily identifiable, emitting smaller branches; we will also see these emitting fine branches, often very long, bearing fructifications identical to those described so far.

Let us now examine the mold formations that we encounter in other very different parts of the rat's organism, in the white matter of the hemispheres and in the choroid plexuses for example.

Figure 1 of Plate 14 shows the shapes of various elements observed in the white matter of the hemispheres and which need not be described, as these shapes are identical to those of the spleen.

There are mycelial filaments bearing oval dilations in strings (1) which are found on the mycelial filaments of *Penicilium* cultivated on liquid media.

There is an enormous mass of small spherical masses and granules similar to mold spores carried at the end of mycelial filaments (4, 5), like mold spores.

Figure 2 of the same plate shows the elements observed in the choroid plexuses. These are:

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1. Elements identical in shape to those already observed in the spleen and the white matter of the hemispheres (2, 4, 5). Some of these elements have the shape of a trypanosome (5).

2. Large germinative masses (sclerotia) emitting large mycelial trunks (1).

3. Hyaline filaments bearing regularly spaced dilations. and, in each dilation, an oval basophilic mass (6). These filaments are obviously fragments of dendrites of brain cells, but some are terminated by a ball resembling a chlamydospore, and identical mycelial filaments are found in cultures of molds of the *Mucor* type.

4. Dilated spindle-shaped filaments (3) emitting at the level of the latter a mycelial filament bearing string-like dilations. Exactly similar formations are found in molds that develop in cultures of the pulp of animal organs.

5. A considerable mass of isolated granulations (0.4 to 1 μm or more) resembling mold spores. Some granulations, identical to these, are borne at the ends of the ramifications of mycelial arborizations which are evidently sporiferous hyphae of molds. Finer granulations identical to zymospores are also noted.

6. Bacterial elements of various shapes (8) and a large number of spirillae; these are (has already been noted previously) spiral appendages of the mold mycelium.

These various elements can all be identified as belonging to molds inhabiting the healthy animal organism; the only exceptions that can perhaps be made, for the moment, are the filaments that are elements of dendrites of brain cells, and the fine granules identical to zymospores, the origin of which escapes current observation.

Wanting to determine whether these formations indeed correspond to mycoses of infection, I looked for them in the liver of the newborn rat. Figure 3, plate 18, is a photograph of a preparation of the spleen of a young rat removed from the mother's uterus. It appears, at first glance, that mold elements do not exist there. Examining the figure with a magnifying glass, the pedicle of a large number of elements can be distinguished. If longer mycelial filaments are missing here, it is because of their extreme fragility.

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Cat. I observed, in the substance of the cerebral hemispheres, elements similar to those already described; Figure 1, Plate 15, shows a large mycelial filament bearing dilations such as are observed in *Mucor* cultures. In addition, there is a large mass of granules

identical to spores, some bearing a vestige of their pedicle and bacillary elements of various shapes.

I observed in the spleen, in addition to the common fruiting forms already described (1, 2, 3), voluminous germinative masses (sclerotia) emitting large mycelial trunks (5, Fig. 2) which are easy to identify, because the partitioning is visible there (4, 5); there is, at each point of partitioning, a dilation (node) similar to those presented by certain mycelial trunks of molds.

Pig. I observed in this animal:

1. In the white matter of the hemispheres: a large quantity of isolated oval masses of 8 to 12 μm, quite strongly stainable by dahlia (1, 2, 3, fig. 1, pl. 16); mycelial filaments bearing dilations occupied by oval masses similar to those scattered in the preparations; examination of certain mycelial filaments shows that these oval masses are, at a given moment, expelled from the filament (3, fig. 1). Similar filaments are observed in *Mucor* cultures; the basophilic masses occupying the dilations are also, at a given moment, expelled from the filament: these are intercalary chlamydospores in both cases.

2. In the grey matter of the cerebral hemispheres (fig. 2): Mycelial filaments giving rise to spores along their path (1, fig. 2); mycelial filaments bearing characteristic chlamydospores (4); mycelial filaments, 2, 3, bearing string-like dilations stuffed with spores, a common arrangement on the mycelium of molds; pedunculated spores, borne in the axils of two dilated terminal mycelial filaments (8); a large mass of granulations identical to those contained in the mycelial dilations and bacterial elements of various shapes.

3. In the white matter of the hemispheres, numerous mold elements already described (Fig. 3).

Ox. In addition to the common elements already described, I observed, in the white substance of the hemispheres, numerous mycelial filaments bearing either string-like dilations (4, fig. 2, pl. 17), or long spindle-like dilations (2, 3, 6), some ending in a ball (1).

In the liver, I observed, in addition to the common elements of molds, the same richness in mycelial filaments bearing string-like mycelial dilations, or large oval dilations (Fig. 1).

Plate 18 contains two photographs (Figs. 1 and 2) of a preparation of beef liver. The mycelial elements bearing dilations (Fig. 1) remained colorless in the preparation, probably because they had undergone fatty transformation.

Dog. In this animal, I observed easily identifiable mold elements in the choroid plexuses and in the white substance of the hemispheres.

In the choroid plexuses, the following can be seen: Large mycelial branches bearing dilations (1, fig.1, pl.19) and emitting lateral ramifications ending in spherical masses equipped with a large basophilic nucleus (2).

Large mycelial trunks (3) emitting lateral branches bearing dilations (2); on these are born characteristic sporiferous hyphae (7).

In the substance of the cerebral hemispheres (Ammon's horn) I observed other elements characteristic of molds.

Mycelial filaments provided with string-like dilations (1, fig. 2, pl. 19) and ending in terminal chlamydospores (11).

Mycelial filaments bearing lateral chlamydospores (5).

Germinative masses emitting a mycelial filament which bears string-like dilations and a terminal spore (4).

Large germinative masses (6) (agglomeration masses), some torn (10) and emptying their contents: spores and zymospores.

Fruiting bodies in the form of spherical or pyriform balls (3) pedunculated and grouped in pairs at the ends of the same filament.

Fruiting bodies of common types already described. These observations were made on the brain of a rabid dog. I had to check them on the brain of normal dogs; the same elements exist there. However, the forming germinative masses marked with number 6 (Fig. 2), as well as the large bacillary elements represented in the center of the figure, are specific to the rabid dog and relate to Negri's corpuscles.

As proof of the mycelial vegetation of molds in the animal organism, I will mention one last observation made on the dog. I wanted to see if injecting this animal with an anti-spleen serum would destroy the molds normally found in this organ. This serum was prepared by intravenous injection of an ethereal extract of dog spleen into a goat. On the sixteenth day after the injection, 20 cubic centimeters of the goat's serum were injected into the dog, which subsequently received three doses, eight days apart.

I do not know if this serum had a noticeable effect. The spleen of the injected dog, which appeared more fibrous than normal, had not increased in weight. But, upon microscopic examination, the mold elements appeared to have taken on a particular appearance and to fix the basic dyes much less. This is indicated only to establish

exactly the conditions of the observation, the importance of which is linked solely, here, to the determination of the existence and form of the mold elements in the organ. Figures 1, 2, 3, 5 of plate 20 show these elements of forms already described; one can clearly see, under a magnifying glass, the segmentation of the mycelial filaments. This segmentation is even clearer in figure 4 where one sees mycelial filament 1 carrying three successive mycelial articles, 2, 3, 4, and article no. 4 emitting three new mycelial branches, the central one of which is terminated by a granular fructification of spherical shape.

The presence of mold elements in all normal organisms is therefore demonstrated by a sufficiently considerable number of observations to remove the slightest doubt. These observations establish that molds vegetate in the organism in the mycelial form and develop, with their mycelium, fruiting bodies identical to those they form in artificial environments and under certain conditions, for example, vegetation immersed in a liquid medium or vegetation at a temperature which, while permitting the development of the mycelial form, is unfavorable to that of the aerial form.

I will describe some types of mold fruiting bodies observed in vitro under these conditions, in order to supplement the observations already made by identifying the fruiting forms observed in the animal organism.

Identification of the vegetative forms observed in the animal organism with those of molds cultivated in vitro.

Along the way, it has just been indicated to which gonidial types of molds should be related the fruiting forms observed in the animal organism.

A first method for identifying all the mold elements observed in an organism can be used successfully: it consists of culturing the tissues, reduced to pulp, on sugar agar, observing the fruiting forms of the gonidial types of molds that develop immediately in the culture, and then following them until their complete evolution. Using this method, I obtained, by culturing the electric organ of the torpedo, a pearly white mold of the *Aspergillus* type which presented, in its first generation, fruiting bodies identical to those that exist in great numbers in the animal organ (See plates 112 and 113).

It is impossible for me to identify all the fruiting forms here; it would be pointless, as it will be done along the way in the studies that follow.

I will only explain the identification of the ball and spindle shapes with those taken by the fruiting bodies of certain hyphomycetes of the *Penicilium* or *Cytromyces* form, when they do not reach full development.

Plate 21 shows the ball (1, fig. 1, pl. 21), pear (4, fig. 4; 5, fig. 5), and spindle (2, fig. 1; 2, fig. 2; 3, fig. 3; 3, fig. 5) shapes that the fruiting bodies of a *Penicilium* can take when they develop slowly or remain rudimentary. This *Penicilium* developed on a fragment of cancerous uterus; its culture presented the forms indicated above in some places where it vegetated in the form of a creamy coating; in others, where it took the form of an aerial hyphomycete, the gonidial stems of *Penicilium* were fully developed and characteristic. There was no doubt that the pear, ball and spindle shapes belonged to the same culture, because in some the formation of the gonidial end and even of the sterigmates was perceived (2, fig. 1).

I was able to obtain the passage of the spindle-shaped fructifications of the *Epidermophyton inguinale* and *Achorion gypseum*, to the *Penicilium* form; the sterigmate groups of the latter form in the spindles.

In many species of *Citromyces*, the terminal group of sterigmates is born in a pear-shaped sac, the envelope of which is rejected after development. The example shown in Figure 7, Plate 21, concerns the fruiting bodies of a mold isolated from the cerebro-spinal fluid of patients suffering from encephalitis lethargica. We observe, side by side on the same mycelial branch, fruiting bodies still included in their sac and others developed into *Citromyces*.

Figure 6, Plate 21, shows ball-shaped formations identical to those found in animals; they existed in a culture of charcoal on broth that spontaneously passed to the *Penicilium* form.

I will confine myself to this demonstration, which is sufficient for the moment, since others will be made during subsequent studies.

At the point we have reached, it becomes necessary to examine the significance of the observed facts. We have observed the presence of a considerable quantity of fungi in the organs of healthy animals. Since these molds exist in all animals of the same species, there is no doubt that they are normal in healthy animals.

We must therefore conclude that there exist, in all individuals of all animal species, permanent, normal mycoses.

This conclusion seemed justified by the existence of certain permanent mycoses: syphilis, for example. But other facts appeared to demonstrate its inaccuracy.

For example, it seems surprising that *animals do not suffer from the presence of these molds,* although their quantity is considerable in the most essential organs.

On the other hand, we note that the gonidial forms obtained in healthy animals, by culturing the pulp of their organs, are related to a single species of mold infecting all animals of the same species.

I have researched and noted the presence of mold elements in the connective tissue as in the various organs. I have even observed them in such great numbers that I wondered if, in this tissue, there exist elements belonging specifically to the animal, because having looked for them again, I was unable to find a single one. I was able to link all the observed elements to molds, without exception;

I was therefore led to this conclusion: *The connective tissue of animals is entirely made up of the vegetation of one or more molds.*

With the knowledge *I had at that time*, the following two notions were obvious:

1. Connective tissue, being formed exclusively by mold vegetation, is of a plant nature.

2. It is therefore a tissue foreign to the organism.

Due to the enormous quantity of connective tissue contained in a mammal's organism and the crucial supporting role it plays, these conclusions seemed inconsistent and unacceptable.

Subsequent observations and the study of the organism's other tissues will allow us to judge otherwise. But, at that time, the need arose for a new, complete study of connective tissue and, if necessary, other apparently healthy tissues of the organism, with a view to establishing, in the latter in particular, a distinction between the elements of mold and the normal elements specific to the organism and assigned to its functioning.

Let us move on to the presentation of this study.

* * * * *

CHAPTER III

STUDY OF THE CONSTITUTION OF TISSUES OF THE ANIMAL ORGANISM

This study will focus on the following tissues, elements, or organs:

1. Connective tissue.

2. Nerve fibers.

3. Fundamental anatomical elements of nerve centers.

4. Motor end plates.

5. Muscle fibers.

6. Cardiac muscle.

7. Liver.

8. Spleen.

9. Ovary and testicle.

10. Figured elements of the blood.

11. Electric organ of the torpedo.

I. Study of the elements existing in the connective tissue of animals.

This study was carried out on the connective tissue of various regions in dogs and cattle, and using preparations made by spreading thin tissue slides dry, rapid desiccation, dahlia staining, or impregnation with silver nitrate.

To simplify the presentation, I have brought together, in a schematic drawing (photographed on plate 22, figure 1), the main elements observed in connective tissue and which will be found photographed in other plates. These elements are:

1. Connective tissue bundles (1).
2. Connective tissue fibers (2).
3. Fixed connective tissue cells (3).
4. Leukocytes.
5. Large granular cells, often devoid of nuclei, called nutrient cells (Mastzellen).

I also noted the presence of elements, some of which are of a nature previously unknown, others undescribed; these are:

1. Large mycelial branches (4) without any apparent fibrillar structure, bearing a large quantity of fine pedunculated spores (5) or fine sessile spores (6).

The nature of these branches is further established by the presence of the chlamydospores they carry.

Plate 24 contains a photograph (Fig. 2) of a mycelial trunk on which we notice numerous points in the process of budding (1) which are the origin of the emission of branches, spores or chlamydospores.

Large mycelial branches of a similar shape are found in the submerged mycelium of *Penicilium* cultivated in liquid media, and in the vegetation of other forms of mold, mainly the *Mucor* form.

2. Chlamydospores (7) which I have just mentioned and other formations (8) of the same nature.

3. Mycelial branches bearing spindle-shaped dilations ending in a point at their two ends (9); mycelial branches bearing cylindrical dilations (10). These various dilations are identical to those found on the mycelial branches of molds.

4. Mycelial branches on which the segmentation into articles is very apparent (11).

5. Fruiting bodies in the form of septate spindles (12).

6. Mycelial branches packed with spores (12) like those of molds.

7. Spore-bearing hyphae (14) bearing fine refractile spores (16) or isolated branches (15) bearing a spore at their tip.

Some of these spore-bearing hyphae or isolated spore-bearing branches branch directly from *the connective tissue bundles, which establishes that these bundles are groups of mold mycelial branches.* On the other hand, by following the ramifications of a large bundle like the one in Figure 1, Plate 22, we can distinguish isolated spore-bearing hyphae or branches branching off from a ramification of this trunk.

8. Germinal masses (1, fig. 1; 1, fig. 2, pl. 23); groups of agglomerated germinal masses which will become sclerotia (2, fig. 1, pl. 23).

9. Chlamydospores or the beginnings of ball-shaped fruiting bodies, sometimes joined in pairs on the short branches of the bifurcated end of a connective fiber (Fig. 4, pl. 22).

10. Masses of agglutinated spores and zymospores which are germinal masses in the process of formation and which will become the elements called nutritive cells (Mastzellen).

On the other hand, decisive proof of the nature of connective tissue is provided by the demonstration:

1. Of the origin of connective bundles.

2. On the nature of fixed connective tissue cells and their relationship to connective tissue bundles.

Origin of connective tissue bundles.

By following the path of the largest connective tissue bundles, we see them reach large masses that are highly stainable with dahlia, which are the sclerotia that give rise to them. These emit several large mycelial trunks that are connective tissue bundles, or rather, that will be when they have divided into a fairly large number of branches remaining enclosed in the same sheath.

If we spread a thin section of intermuscular lamellar connective tissue from beef on a glass slide, we observe, after staining with dahlia and examining it at low magnifications (50 to 250 D), numerous sclerotia emitting bundles.

Plate 23 shows three photographs of such preparations. Figure 3 contains numerous sclerotia, of which I have marked only the easiest to distinguish with a cross. Figures 4 and 5 show an enormous sclerotium at two different magnifications. In Figure 5, the image has been underdeveloped so that the enlarged sclerotium and the bundles emanating from it appear alone. In Figure 3, two large bundles emanating from the largest of the sclerotia marked with a cross are clearly visible, one at each end.

In the mold preparations, identical sclerotia, equally large, are distinguished, also giving rise to large mycelial trunks.

Nature of the fixed cells of the connective tissue and their relationship to the connective bundles.

The fixed cells of connective tissue are dilations placed on the path of mycelial branches emanating from the connective tissue bundles. The flat cells, included in the thickness of the bundles, are, like these, dilations placed on the path of the fibers. These conclusions are supported by the following facts:

1. If we examine a large connective tissue bundle at the points where it is about to divide, and where it is sufficiently transparent, we see the flat cells placed on the path of the connective tissue fibers, of which they are dilations (Fig. 3, pl. 22).

2. If we follow the connective tissue bundle when it dissociates or one of its branches dissociates, we find the flat cells (1, 2, 3, 4, 5, fig. 4, pl. 24) on the path of the fibers.

3. We also find, along the path of the fibers, dilations of all sizes (9, 9, 9, 9, fig. 1, pl. 22) of the same shape as the fixed cells. These are fixed cells in the process of formation.

We often observe, on the same filament and placed one behind the other, several dilations whose size gradually decreases. *This arrangement is also observed along the path of the mycelial filaments of molds cultivated in vitro.* Plate 25 shows these mycelial dilations in an in vitro culture of the *Mucor* form from a culture of human tuberculosis.

4. If we examine, in a state of complete development, the fixed cells scattered in the connective tissue, we observe at each end of many of them a very long filament (connective fiber) which extends them (Fig. 2, pl. 22; fig. 3, pl. 26). By following these filaments, we always manage to observe some which emanate from another fiber or from a connective bundle.

5. When sufficiently developed, the dilations of the mycelial filaments of the molds themselves come to play the role of sclerotium. The fixed cells of the connective tissue also play the role of sclerotium and emit mycelial branches (1, 2, fig. 1, pl. 26).

6. Certain fixed cells (1, 2, fig. 4, pl. 26) are extended by a filament carrying a spore or chlamydospore.

7. A certain number of fixed cells which emit four or five mycelial filaments (1, 2, fig. 1; 1, fig. 2, pl. 26) have an appearance identical to that of certain cells of the nerve centers (pyramidal cells, cells of the plexiform layer, polymorphic cells, cells of Retzius, Cajal, etc., of the cerebral cortex).

We now know the main elements of connective tissue: large, medium, and small bundles, various fibers, fixed cells, and the relationships they have with each other; we know that the bundles arise from germinative masses, sclerotia, spread throughout the fabric of the tissue. We have already also noted the presence of other elements whose nature has been explained.

The following observations, made after the previous ones on the intermuscular lamellar connective tissue of the ox, will complete them and show us some new elements.

1. Presence of germinative masses in connective tissue.

We have seen that large connective tissue bundles emanate from large germinative masses or sclerotia. In the intermuscular lamellar tissue, dissociated, stretched, and well spread dry on a glass slide, we observe in certain clear areas numerous germinative masses of various shapes.

A. Some are granular, devoid of basophilic protoplasm (K, fig. 3, pl. 27); their size is very variable (10 to 30 or 40 μ). These are the cells called mastzellen or nutritive cells; they play no nutritional role; they are clusters of hyaline spores formed on the connective tissue fibers and detached from their support;

these clusters represent a first stage in the formation of the germinative mass that we find in the following types, which differ in the basophilic elements that have joined them.

B. Others are entirely basophilic and emit very long mycelial filaments (A, B, C, D, E, F, G, fig. 3, pl. 27); they are one of the origins of connective fibers; these are born on germinal masses of all sizes.

C. Others (H. J, fig. 3, pl. 27) have the appearance of white blood cells but are much larger (20, 25 μ). They are made up of hyaline spores to which basophilic elements have joined, the protoplasm of which is distributed throughout the mass in one or more nuclei. At a given moment, they emit connective fibers.

There is actually no difference between these various elements; they are germinative masses at various stages of their formation and of various sizes; the size of the germinative masses, which can vary from 3 to 100 μm or more, has no relation to their constitution, which remains the same.

It is interesting to examine Figure 3, Plate 27, with a magnifying glass. We notice a large quantity of spherical granules, some of which are carried at the end of a short pedicle adhering to the connective fiber from which it emanates. These granules are spores formed by the connective fibers, which are in reality mycelial filaments.

Figures 1, 2, 4, 5, plate 27, allow us to observe under a magnifying glass this same formation of spores on the mycelial filaments of several molds: that which forms the *Eberth bacillus* (Fig. 1), a second from a tetanus culture (Fig. 2 and 4), a third found in the blood of patients suffering from scarlet fever (3, fig. 5).

This examination shows the perfect identity between the mycelial and connective elements. Figure 1 represents the transformation, on agar, of a culture of typhus bacillus;

the spherical germinative masses emit the fine ramified mycelium which has been described under the name of cilia of *the typhus bacillus.*

Large, often enormous, germinative masses are also observed in the intermuscular connective tissue. Several are shown in Figures 4, Plate 29, and 3, 5, 6, 7, 8, Plate 30. They are seen emitting either large mycelial bundles (Fig. 5 and 8) or fine connective fibers (Fig. 7).

2. Division of connective fibers into fibrils.

A small or medium-sized connective fiber often branches out, without any apparent ramifications, into a considerable number of smaller fibers (A, Fig. 1, plate 28) or wavy fibers, often twisted into spirals. Thus, in Figure 1, Plate 29, a fiber B branches out into a large bundle of spiral and wavy fibers A. Often, such a bundle reconstitutes later and returns to its original size. This is probably an artificial dissociation caused by the preparation manipulations, because it stops at the rings carried by the bundles (C, Fig. 1, plate 29).

3. Presence of varicosities or mycelial dilations on the fine connective fibers.

Figure 1, Plate 29, contains a large number of fibers with these dilations or varicosities, particularly in the F regions (examine with a magnifying glass). Identical varicosities are observed on the fine mycelial filaments that arise on the large mycelial branches of mucor-type molds.

4. Hyphomycete fructifications of connective tissue.

Large stalked spores (Fig. 3, Plate 29) with a nucleus that is stained more strongly blue by the dahlia than the rest of the spore are easily observed in the connective tissue. Examining the interior of these spores (A, B, for example) under high magnification, we see that they are made up of a small arborization formed by the ramifications of the end of the peduncle.

In the same figure, examined under a magnifying glass, we will find numerous small spores, each borne on a short peduncle emanating from a branch of a connective fiber. Such spores are seen in most other figures.

5. Formation of fruiting bodies in the connective tissue by twisting or coiling of the ends of the filaments.

Twisting or coiling of the ends of mycelial filaments is known to be one of the modes of formation of perithecia in molds. Figures 5 and 6, plate 29, contain several types of these coilings of connective fibers, either twisting (A, fig. 6) or coiling (A, fig. 5).

In Figures 1, 2, and 4, Plate 30, there are masses consisting of fine connective filaments coiled into a compact ball; this is a mode of formation of germinative masses similar to the previous one.

Figure 7, Plate 29, deserves special mention. It represents a connective fiber bearing fruiting bodies typical of *Mucor racemosus*. The photograph was taken from a smear preparation of one of the fibrous tracts extending from a breast cancer the size of an orange. The portion of fibrous tract examined was about 5 centimeters from the tumor, but was surely already contaminated, given the size of the tumor.

I have compared this type to the fructifications represented in Figure 8, Plate 29 (intermuscular connective tissue of the ox); we will see, at A, a filament bearing fructifications of the same kind, but minute (monosterigmate gonidial type).

6. Interannular constrictions.

The trace of mycelial partitioning is found on the connective fibers. Figure 2, Plate 29, shows a connective fiber that has retained the trace marked by the two transverse lines A, B. It will be demonstrated later that these lines are equivalent to the interannular constrictions of the nerve fibers.

7. Spiral windings of the fibers.

The connective fibers, often wavy or spiral, even within the connective tissue bundles, frequently wind around the bundles, even very large ones, or fine fibers. This arrangement, particularly frequent in intermuscular connective tissue, is also found in regions of the body, even in the pia mater where it is very common.

Plate 31 shows multiple forms of this winding, which I will return to in more detail when studying muscle tissue.

8. Appendages of the fine connective fibers.

By examining connective tissue preparations heavily impregnated with silver nitrate at high magnification (1100 to 1500 D), we see that the connective fibers end, after multiple ramifications, in sinuous, wavy branches bearing:

A. A multitude of small branches rolled into spirillae, these often ending in a small round spore.

B. A multitude of short or more or less long appendages, of variable shapes, very often swollen in the middle, like the isolated sterigmates generally carried in vitro by mycelial filaments of the *Sterigmatocystis* type. These appendages are of the same nature as those carried by the dendrites of the pyramidal cells of the cerebral cortex.

This mode of fiber termination is identical, as we will see in the course of this work, in all the tissues of animal organisms and in all the tissues of plant organisms; it is also the same in the finest ramifications of molds cultivated in vitro.

If we don't see it at first glance in a preparation of *Mucor*, for example, it's because we are not looking for it where we should. It is this multitude of spiral branches and appendages which, intertwined with each other, constitutes the external membrane of the mycelial branches of *Mucor*; the mycelial branch itself, at least in the young or adult state, is filled with branched filaments in this form.

The form of this vegetation can easily be seen by examining Figure 2, Plate 28, and Figs. 1, 2, 3, 4, and 5, Plate 31, under a magnifying glass.

Nature of the adipose cells of connective tissue.

I will conclude this presentation with some observations on adipose cells. Fat can form and accumulate in all elements of connective tissue, particularly in fixed cells and in large mycelial trunks and their dilations; Figures 3 and 4, Plate 31a, show fixed cells that have become fatty.

But, in general, the cells that are assigned to the accumulation of fat, or undergo it, are originally spherical or pear-shaped masses, arising along the mycelial filaments to which they are attached by a short pedicle. Figure 1 shows these formations along connective mycelial filaments where they take on the appearance of a *Mucor* mycelial filament bearing numerous chlamydospores.

Some cells remain single and keep their shape, but others elongate and form a sac constricted at several points by outer circular mycelial filaments; therefore, many fat cells are made up of several balls placed end to end.

When the number and volume of fat cells becomes considerable, their bags, as well as the mycelial filaments that strangle them, present the appearance (Fig. 2, pl. 31 bis) of

a plant parenchyma with its supporting carcass (See: *Constitution of plant tissues*), and in particular the appearance of the parenchyma of a potato or a fruit.

Each of the fat cells, single or multilocular, is formed by the division of its mycelial pedicle into multiple branches which form an arcuate tissue in the interior of the bag and, at the periphery, the membrane of the bag by spreading certain branches on a single plane.

This study of connective tissue mentions all the elements that were observed there. All can be identified as mold elements. No other elements were encountered there.

The connective tissue is therefore entirely made up of mold vegetation, and it does not contain any elements other than those of these molds.

By cultivating it in vitro, we should therefore see them appear in the usual form of hyphomycetes. This is what the experiment verified. Cultivating the connective tissue of animals gives rise to several gonidial types of hyphomycetes.

Plate 32 shows the molds obtained by culturing either the loose subcutaneous connective tissue of the dog (Figs. 1 and 3), or the nervous substance of the spinal cord (Fig. 2), or the layer of leucocytes which covers the blood corpuscles after centrifugation of the blood. Plate 33 shows the molds obtained by in vitro culture of the intermuscular connective tissue of the same animal. Examination of these photographs under a magnifying glass is demonstrative; we find there the spores which carry the connective fibers and, in Figures 4, Plate 32, and 5, 6, Plate 33, the fruiting type observed in the connective tissue of the ox (Fig. 8, pl. 29).

The formation of these molds on connective tissue cultivated in vitro, even under gonidial types that are found in the tissue itself, therefore confirms the conclusion drawn from the observations.

We are faced with a conclusion whose accuracy is certain, obvious, but whose consequences are unacceptable. Indeed, if the connective tissue is made up entirely of mold growth, then mold growth must also exist everywhere this tissue penetrates, that is to say:

1. In all organs.
2. Around the nerves for which the connective tissue constitutes a sheath.
3. Around the vessels (vessel sheath) and against or in the wall itself.
4. In the periosteum and bone marrow.
5. In the fibrous envelopes, capsules, surrounding the organs.
6. In the meninges, the dura mater, and the pia mater, which are *entirely* made up of the tissue of apparently parasitic molds.

I have verified the accuracy of this conclusion, and this verification has not placed any restrictions on the previous conclusions.

We can infer by induction that, if connective tissue is a parasitic tissue, it would not be possible to rid the animal organism of it, because the entire connective frame- work would disappear; the liver and kidneys, for example, would no longer have a capsule or ligaments to support them and would become floating in the abdominal cavity. Such a removal being impossible and the supporting role played by connective tissue being obvious, we would therefore have to conclude that it is parasitic in nature, but adapted by symbiosis to a function and, consequently, indispensable.

Such a symbiosis seems very dubious, because the parasitic molds that vegetate in the body always have a pathogenic action that cannot be doubted and which is expressed, for example, by the known and distant symptoms of syphilis, by the paralysis and other nervous symptoms that appear after the apparent recovery from certain diseases, diphtheria, scarlet fever, cerebrospinal meningitis, etc.

There was an enigma here because determinism was still insufficient and it was necessary to complete it with the study of other tissues of the organism. It seemed to me that this study should begin with that of the characteristic fibers which constitute the large nerves like the sciatic: the myelin nerve fibers.

II. Nature of Myelinated Nerve Fibers.

From the beginning of this study, I already had a strong presumption that myelinated fibers are mycelial filaments of molds, due to the description by Retzius and Nageotte of the myelinated fibers of the nerve of the exopodite of the antenna of *Paloemon serratus*, fibers identical to mycelial filaments of molds.

Here, no doubt can remain. The branched myelinated fiber represented in Figure 37 of Nageotte's book **(1)** is a mycelial filament of a mold growing in the gonidian form of *Mucor racemosus.*

Figure 1 of Plate 34 represents the photograph of a mycelial branch, B, of the *Mucor racemosus* form of a cholera vibrio (Bologna vibrio cultivated at the Pasteur Inst.) identical to the branched nerve fiber depicted by Nageotte. Several of the constrictions (C) described can be seen.

In addition to this particular branched form, the nerve fibers of crustaceans can be identified with the mycelium of *Mucor racemosus* forms:

(1) *J, NAGEOTTE, l'Organisation de la matière, Alcan, 1922.*

1. By the ribbon-like, non-cylindrical, shape of the nerve fibers studied by Nageotte**.** This is the flattened, ribbon-like shape of certain forms of the mycelium of *Mucor racemosus* (1, fig. 4 and 5, pl. 34; 1, fig. 1 and 4, pl. 35).

2. By the fact that the constrictions do not participate in this flattening and remain cylindrical (Nageotte) (See the figures indicated above).

3. By the fact that the myelin covers the nuclei of the sheath (See also the same figures and in particular figures 4 and 5, pl. 34). These nuclei are the intramycelial spores or intercalary chlamydospores intended to be expelled like any other species of spore. Being of intramycelial formation, the external wall of the mycelial tube naturally covers them.

4. By the special shape of the *rings of Ranvier* in these fibers, indicated by Nageotte (B and C, fig. 39, J. Nageotte) **(1)**. This shape is, *down to its smallest details*, down to the folds of the mycelial wall starting from the constrictions, that of the mycelial constrictions and the intercalary chlamydospores formed at their level in certain forms of *Mucor racemosus*; there will be no doubt in this regard, after examining the mycelial constrictions in Figure 1, Plate 35, and in particular those in Figure 4, Plate 34, photographed at a higher magnification.

Let us add to these facts that the fiber represented by Nageotte in his book (Fig. 37) also presents, to identify it, varicosities (usual mycelial dilations) and, at the ends of the ramifications, *series of balls* that Nageotte believed to be artificial; they often exist on the terminal mycelial filaments of molds of the *Mucor, Aspergillus,* and other types.

Thus, the motor nerve fibers of crustaceans, described by Retzius and Nageotte, are mycelial filaments of the gonidian form *Mucor racemosus* of a mold.

The interannular constrictions that these authors described are mycelial constrictions and intercalary chlamydospores.

Is it the same for nerve fibers and their constrictions in vertebrates?
To examine the nerves of vertebrates, I used the following procedure:

A one-centimeter-long piece of a rabbit's sciatic nerve is sectioned. It is divided longitudinally into five or six fragments and each of them is applied to a very dry and degreased microscope slide. With two needles, the nerve fragment is dissociated and spread over the largest possible surface. The nerve, remaining very adherent to the dry surface of the glass, spreads out by traction, in a very thin section torn in many places *in which numerous dissociated, isolated, and very long fibers are easily found.*

(1) *J, NAGEOTTE, l'Organisation de la matière, Alcan, 1922.*

They are dried quickly at 80° or 90° on the heating plate and stained for five to ten minutes at the same temperature with the dahlia. They are then washed in hot water. By operating in this way, here are the first observations I made on the rabbit sciatica: I observed the following elements:

A. Known myelinated fibers with constrictions of Ranvier.

B. Myelinated fibers along the path of which I did not notice any constrictions or incisures.

C. Bare fibers devoid of myelin. Among these fibers are all those observed in the connective tissue and the medullary pia mater, with the same shapes and the same characteristic fructifications.

D. Fibers without myelin, provided with elongated oval nuclei, identical to those found in the spinal cord and brain and which most often form the dendrites of pyramidal cells.

E. Germinal masses of different shapes and sizes (multipolar, unipolar, and bipolar cells) emitting mycelial filaments that join the mass of nerve fibers and run parallel to them.

Elements C, D, and E are mycelial filaments and sclerotia of mycelium that are the easiest to identify using the characteristics they bear, which I have described several times so far.

Elements C, D, and E are Remak fibers of two different types.

Their identification is the same as that of connective fibers. Figure 264 of Ranvier's *Treatise on Histology* shows a representation of a Remak fiber bearing the string-like dilations of mycelial filaments.

Elements A and B, myelinated fibers with or without constrictions, are mycelial filaments of molds vegetating in the *Mucor* form or in an intermediate form between the *Aspergillus* form and the *Mucor* form.

Here is how I arrived at this identification:

I had already noticed for a long time the striking resemblance that existed between the myelinated nerve fibers of mammals and the mycelial filaments, notably the gonidiophores of *Aspergillus* and *Mucor.*

These filaments, stained with dahlia, show a central cylinder formed of basophilic protoplasm, strongly stained blue, surrounded by a sheath of non- or barely stainable, highly refractile substance; they present the beginnings of mycelial partitioning or seg-

mentation in the form of constrictions reminiscent of the shape of the constrictions of Ranvier.

By comparatively studying the myelinated fibers and the mycelial filaments of *Mucor* and *Aspergillus*, I noticed:

1. That the constrictions of Ranvier do not all have, in myelinated nerve fibers, the known classical form and in particular the form so well described by Nageotte **(1)**. These constrictions have very varied forms; their number and spacing are also very varied. On certain fibers, none are observed over a great length.

2. That the mycelial filaments of *Mucor* and *Aspergillus*, particularly the gonidiophores, bear constrictions strikingly resembling the constrictions of Ranvier. As in myelinated nerve fibers, different forms of these constrictions are found. *On certain mycelial filaments, the constrictions have so many of the known classical forms that, if these filaments were placed side by side with myelinated fibers, it would be impossible for an uninformed histologist not to mistake them for nerve fibers.*

This fact is made very clear by examining Plate 40 containing photographs of nerve fibers, on the left, and of *Mucor* mycelial filaments, on the right. The constrictions of the mycelial filaments of *Mucor* and *Aspergillus* are the points of partitioning of the mycelial filaments.

While making these observations on the constrictions, I noticed at the same time that the mycelial filaments of *Aspergillus* bear, depending on their size, age, or development, the Schmidt-Lanterman network, in the different forms described by Nageotte **(1)**,

These observations having been made, I found on the myelinated fibers of the rabbit and calf sciatic nerve new characteristics allowing them to be identified. I first noticed, on certain fibers, very fine and very short ramifications bearing at their end a small refractive spore, then, at the end of certain large myelinated fibers, a large terminal fructification obviously homologous to the fructifications carried by the gonidiophores of *Mucor* and *Aspergillus.*

I also found numerous fine, highly ramified lateral branches, arising on the wall of the myelin sheath of the fibers.

Finally, I managed to find, at two or three points, a myelin fiber clearly dividing into two or three branches. From that moment on, there could no longer be the slightest doubt in my mind. Myelinated nerve fibers are mycelial filaments of the *Mucor* type.

(1) *J. NAGEOTTE, The Organization of Matter.*

Continuing my study to convince myself even more, I found a new characteristic which, in itself, is sufficient to definitively identify myelinated fibers. On the mycelial filaments of *Mucor* and *Aspergillus*, there is an enormous quantity of fine spiral filaments which, when detached, constitute the spirillae.

On the myelinated fibers of the calf sciatic nerve, I was able to observe the existence of this multitude of spirillary filaments.

Then examining the rabbit's sciatica, I made the same observation.

This set of characteristics, all common to the mycelial filaments of *Mucor* and *Aspergillus* and to the myelin fibers, constitutes a body of evidence such that no doubt can remain about their nature.

I will now present in succession all these characteristics which demonstrate, beyond the slightest doubt, that the myelin fibers are mycelial filaments of molds. The demonstration is given by the following photographic documents.

1. Myelin sheath and cylinder-axis.

In preparations of various mucors, we find mycelial filaments whose appearance is identical to that of myelin fibers. Figure 1, plate 36, represents the photograph of mycelial filaments of *Mucor (Mucor truchisi,* Lucet). Filament B is strictly identical to a myelin fiber It shows the myelin sheath with its characteristic Lanterman network and we can see, on the right, the axis cylinder by transparency. Filament C shows the axis cylinder better; its Lanterman network has partly disappeared because the mycelial tube is older. Tube C, even older, shows on the myelin sheath the polygonal mesh network designated by Nageotte, neurokeratin network. This network has this polygonal shape because it is now formed only by the largest branches emanating from the axis cylinder which come, by their fine ramifications, to form a felting constituting the myelin sheath.

The basophilic protoplasm gradually disappears from the mycelial tube as it ages, the Lanterman network also gradually disappears with the age of the filament; the sheath of the filament becomes discolored and the axis cylinder itself eventually disappears.

In the nerve, the same facts are observed. Some fibers no longer have, or almost no longer have, a Lanterman network, and some no longer contain an axis cylinder.

Figure 2 of the same plate represents mycelial fibers from a mucor obtained by culturing the medulla oblongata of a rabid dog. The axis cylinder and the myelin sheath are also visible.

2. Flow of myelin.

It is known that myelin flows out of the ends of ruptured fibers. Fig. 2, plate 37, shows the flow of myelin from the ends of a myelinated fiber from the rabbit sciatic nerve.

The same occurs for torn *Mucor* mycelial filaments. Figure 1 of the same plate shows the flow of basophilic protoplasm from the ends of two ruptured mycelial tubes of the *Mucor* form of the pathogen of icterohemorrhagic spirochetosis.

3. Transverse striations of nerve fibers.

In addition to interannular constrictions, myelinated fibers exhibit transverse striations. Figure 3, Plate 37, shows an interannular constriction A, and transverse striations B.

The same striations are found on certain *Mucor* mycelial filaments. Figure 5, Plate 37, represents a mycelial filament of the *Mucor* form from a bacterial culture of bovine tuberculosis.

Figure 6 represents a mycelial filament of the *Mucor* form of the pathogen of icterohemorrhagic spirochetosis. Figure 4 represents a mycelial filament of the rabbit sciatica which has lost all the characteristics of myelinated fibers and presents the same appearance as the mycelial filaments in Figures 5 and 6.

4. Lanterman Network.

I have already shown this on the *Mucor* mycelial filaments in Plate 36. It is also visible on *Mucor* filaments in other plates.

5. Schmidt Lanterman Notches.

Figures 5 and 6 of Plate 38 show the notches of the rabbit myelinated fibers.

I found the same incisures in the mycelial filaments of *Mucor,* but they are quite rare or, rather, it is difficult to highlight them. Figure 2, plate 38, shows very close incisures on a gonidiophore, and Figure 3 shows these incisures on a mycelial filament of an indeterminate *Mucor* cultivated by Lucet. But I observed them with all their characteristics on the mycelium of *Mucor Truchisi*-Lucet.

Figure 1, plate 38, represents two incisures of a mycelial filament of this *Mucor*. Examining the edges of incisure A with a magnifying glass, we see that they are connected, as in the incisures of myelin fibers, by fine mycelial filaments.

Examination under a magnifying glass of these incisures, as well as those of the myelin fiber in Figure 5, particularly incisure A in this figure, shows that they are made up of mycelial filaments which, starting obliquely from the axis cylinder, divide into fine branches in contact with the myelin sheath. Careful observation shows that it is these which, through the intricacy of their fine ramifications, constitute the external wall of the mycelial filaments of *Mucor* or of the myelin sheath of nerve fibers.

The axis cylinder of myelin fibers is not always of uniform size in the points where the fibers do not present constrictions. Often, it presents a series of oval swellings in irregular chains.

The same swellings are observed, much more pronounced, in the mycelial filaments of *Mucor* cultured in vitro. These swellings are closely related to the incisures. The starting point of the incisures on the sheath coincides with the point where the edge of a swelling of the axis cylinder comes into contact with it.

Figures 3, 4, 5, and 6 of Plate 39 show the various shapes of the swellings of the axis cylinder in the mycelial filaments of the *Mucor* form of the pathogen of icterohemorrhagic spirochetosis.

In Figure 1 of this plate, a mycelial filament of the *Mucor* form from a culture of human tuberculosis bears a series of swellings very close together in strings, a shape quite common in the myelinated fibers of the sciatic nerve, for example.

Figure 2 shows swellings of the same kind as those in Figs. 3 and 6, in the myelinated fibers of the sciatic nerve of the calf.

6. Interannular constrictions.

In Plate 40 you will find: Photographs of the various forms of constrictions of the myelinated fibers of the rabbit in Figures 1, 3, 5, 7, 9, 11, 13, 17, 19. Photograph of a constriction of a myelinated fiber of the sciatic nerve of the calf (Fig. 15);

Photograph of the forms of interannular constrictions in the mycelial filaments of *Mucor (Mucor Truchisi*, Fig. 20; *Mucor-Regneri*, Fig. 18; *Mucor* form from a culture of human tuberculosis, Fig. 14; *Mucor* form obtained by culture of the medulla oblongata of a rabid dog, Figs. 2 and 12; *Mucor* form of the agent of icterohemorrhagic spirochetosis, Figs. 4, 6, 8, 10, 16).

On the other hand, in Plate 41, we will find photographs of the interannular constrictions in the mycelium of *Aspergillus glaucus* (var. repens. de Bary) (Figs.1, 2, 3, 3, 4, 5) and in the mycelium of *Mucor mucedo* (Figs. 8 and 9).

The identity between these two species of elements is such that, comparing certain forms on the left side of Plate 40 with others on the right side, an uninformed observer would be unable to indicate which are the nervous elements.

By examining these various figures, we realize that the interannular constrictions of the myelin fibers and those of the *Mucor* myelium are identical and both represent the points of partitioning of the mycelium or, again, the points of junction of the different mycelial articles.

This identity continues even in certain particularities, such as:

A. The presence of a stalked spore arising at the point of constriction. This particularity exists in a mucor constriction (Fig. 8) and in a myelin fiber (Fig. 7).

B. The presence of a cone starting from the constriction and connecting to the axis-cylinder that it marks at this level. This cone appears to be constituted by the myelin sheath of the opposite mycelial segment which is inserted onto the axis-cylinder or, rather, it is the axis-cylinder which, at the level of the apex of the cone, emits fine ramifications which constitute the conical membrane further constituting the myelin sheath of the following segment.

This cone can be seen: figs. 2, 6, 8, 10, plate 40; figs. 8, 9, plate 41, on the mycelium of *Mucor*, and figure 5, plate 40, on a myelin fiber of the rabbit sciatic.

C. Figure 1, Plate 41, shows a mycelial filament of *Aspergillus repens*, whose ruptured outer sheath reveals the persistent axis-cylinder. This identical arrangement can be found in a figure in Ranvier's *Treatise on Histology* relating to myelinated nerve fibers.

We can therefore see that the myelinated fibers are mycelial filaments of *Mucor*, that their interannular constrictions are the junction points of the mycelial articles and that, consequently, these constrictions are in no way intended to nourish the fiber as had been supposed.

If the myelinated fibrins take on a more vivid color at the constrictions, it is not because the nerve is more permeable at this point as has been said, because this same peculiarity exists *for all varieties of molds at the points of partitioning;* this is because at this point, there almost always remains, localized, a certain quantity of basophilic protoplasm.

Moreover, this more vivid coloration is observed on certain myelin fibers whose partitioning, barely indicated, is not marked by a constriction. Figures 6 and 7, plate 41, represent two myelin fibers from the rabbit sciatic, stained with dahlia, which are in this case. Figure 6 shows a myelin fiber with a well-formed constriction, while the other is visible only by the persistence of basophilic protoplasm at the point of partitioning.

7. Nuclei of the Schwann Sheath.

These nuclei are described as being those of the sheath attached to its external face. These are not sheath nuclei. They are spores placed at the end of a very fine peduncle arising on the myelin sheath, often quite far from the spore, or, sometimes, intercalary spores of a filament arising on the myelin sheath and extending further than the spore.

Figure 3, Plate 42, shows a myelinated fiber from the calf sciatic nerve bearing a Schwann nucleus at the very level of a constriction, which already shows that it is not a sheath nucleus. This nucleus is an elongated intercalary spore contained in a varicosity of the mycelial filament A, which continues at B, beyond the spore, and which originated lower down, on the Schwann sheath itself.

Ranvier gave in his *Treatise on Histology* a figure (Fig. 257) showing a similar nucleus which he calls the nucleus of the interannular segment; in reality, this nucleus is identical to the one photographed on plate 42, figure 3. In fact, we see there a part of the mycelial filament containing the nucleus and which Ranvier confused with protoplasm surrounding the nucleus. Figure 3, plate 42, leaves no doubt that this nucleus is contained in the mycelial filament A, distinct from the myelin fiber and born on its Schwann sheath at a more distant point.

In Figures 1 and 4, Plate 42, pedunculated Schwann nuclei (A, B, C, D, Fig. 1; A, B, Fig. 4) will be seen on the myelinated fibers of the rabbit sciatic nerve; this arrangement shows that these nuclei are spores, and *that it is because they are borne on peduncles emanating from the Schwann sheath that they are external to this sheath.*

These nuclei are found, in an identical arrangement, on the mycelium of *Mucor.* Figures 2, 5, and 6, plate 42, show these stalked spores on the mycelium of *Mucor* that I isolated from the medulla oblongata of a rabid dog, and figure 5 on the mycelium of a corymbose *Mucor.*

8. Fructifications of Myelinated Fibers.

All the typical fructifications of in vitro *Mucor* cultures are found in the nerves, on the myelinated fibers themselves or on the branches they emit. These fructifications are so characteristic that they alone would establish the identity between the myelinated fibers and the mycelial filaments of *Mucor.*

Plate 43 shows, photographed, the various fructifications that are found on the myelinated fibers of the rabbit. Figure 2 shows, in A, for comparison, a normal fruiting body of a *Mucor* obtained by transformation of a culture of human tuberculosis.

Figures 1 and 3, plate 43, show two myelinated fibers, one large, the other much smaller, bearing at their ends a fruiting body characteristic of Mucor (A, fig. 1; A, fig. 3). Figure 4 shows, in A, a spore head from which the cap B has detached, and, in C, another fragmented head. Figure 6 shows a spore head A, of which the rounded central part is clearly visible, as in the spore heads of *Mucor (columella)*; this fruiting body is still borne on the myelinated fiber D, which it terminates, and the debris of the cap or capsule still exists in B and C.

In Figure 5, Plate 44, we see three other spore heads, one of which, A, is still attached to the myelinated fiber that it terminates.

In Figure 1, Plate 44, in the middle of a large mass of undissociated myelinated fibers, three spore-bearing heads can be seen.

Figure 3, Plate 44, contains two other terminal fructifications of the same kind.

One might think that this is not the characteristic fructification of *Mucor*, but germinative masses emitting myelinated fibers. This is not the case, because the gonidiophore of the most characteristic fructifying mass (A, Fig. 1, Pl. 43) no longer possesses basophilic protoplasm except near this mass, *which indicates that this is indeed the termination of the fiber and that the mass that terminates it is a fructification.*

In Figure 4, Plate 44, we see a branch C of a myelinated fiber bearing two oval shaped terminal fructifications, A, B. Similar fruiting bodies are found in connective tissue and in the interstices of muscle bundles.

Figure 5, Plate 43, shows a remarkably clear lateral chlamydospore A developing on a myelinated fiber B of the rabbit sciatic nerve. Fig. 7, Plate 43, represents two chlamydospores of the same genus, developed on two mycelial tubes of a mucor isolated from the brain of a rabid dog, and Figure 8, Plate 43, a pedunculated chlamydospore A, from the same mucor. Pediculated chlamydospores identical to this chlamydospore A are

found on the ramifications of the myelinated fibers, and some are frequently found detached from their pedicle in the field of the preparation.

9. Spiral appendages.

Fig. 2, Plate 44, shows some interesting features; first of all, the longitudinal striation of certain myelinated fibers, a striation identical to that of the mycelial filaments of *Mucor*. This striation is due to fine mycelial fibers bearing laterally an innumerable quantity of fine appendages ending in a spore and other appendages wound in a spiral (spirilla). It is these filaments, their ramifications, and the appendages they bear which, by their intricacy, constitute the Schwann sheath.

The sarcolemma of muscle fibers and all membranes, in general, have the same constitution.

Between the myelinated fibers, there are very small germinative masses from which radiate a certain number of fine filaments covered with spiral and spore-bearing appendages. These formations are found identically in the brain and spinal cord, where they constitute the neuroglia. The neuroglia cells are small germinative masses giving rise to branched mycelial filaments with an irregular, sinuous course and covered with spore-bearing and spiral appendages.

The spirillary and spore-forming appendages, which exist on myelinated fibers, also exist on their finer ramifications. Plate 45 shows (Figs. 2, 3, 5) these appendages on rabbit myelinated fibers of various sizes or on their ramifications (branches).

These appendages exist, in an identical form, on the mycelial tubes of *Mucor* and within them; they are also found on the mycelium of *Sporothricum* forms and on other gonidian forms.

Figs. 1 and 4 show them on the mycelium of *Mucor truchisi* (Lucet), of which they form the external membrane, homologous to the Schwann sheath. It will be noted in these figures what perfect identity exists between a myelin fiber and a mycelial filament of *Mucor,* not only from the point of view of the appendages, but also in relation to the aspect of the myelin sheath and the axis-cylinder.

Figure 2, Plate 46, shows the same appendages on the mycelium of a gonidial form of *Sporothricum*. This *Sporothricum* (transformed in another culture into *Penicilium*) was isolated from a teratoid dermoid cyst of which it was the vegetative agent.

It is the spiral appendages of the mycelium which, when detached, constitute the spirilla or spirochetes that have been considered protozoa, agents of certain diseases. Example: *Treponema pallidum.*

10. Branching of Myelin Fibers.

Contrary to what histology treatises teach, myelin fibers branch like connective fibers and like all mycelial filaments of molds.

Fig. 1, Plate 46, shows the ramifications of the end of a myelin fiber A, and a ramification B of the myelin fiber C, which I was able to follow quite far, beyond the point of bifurcation.

Figure 3, Plate 51, shows, with the greatest clarity, the mode of terminal ramification of myelin fibers by division of the axis-cylinder.

In Figure 4, Plate 46, we see a thin branch A, arising from the myelin sheath of a large fiber, immediately branching out into spiral filaments.

Figure 3, Plate 46, shows the division of a myelin fiber C into two branches, A and B. Figure 3, Plate 45, concerning the same fiber, is a photograph taken with a different focus and showing that branch A immediately branches out into spiral filaments.

In Fig. 1, Plate 47, a myelin fiber B, from the rabbit sciatic, gives rise to a branch A on a projection of the myelin sheath; this arrangement is found identically in the neighboring Figure 2 which represents a mycelial filament B of *Mucor corymbife*r giving rise to branch A.

The branches of the myelin fibers very frequently take the form of filament A (rabbit sciatic) divided into two filaments B and C.

Filaments A and C bear, closely packed and pressed against each other, spores D, D, carried at the end of a short peduncle emerging from the Schwann sheath. These filaments are found, in an identical form, in the brain and spinal cord where they constitute the dendrites of the pyramidal cells and the arborizations of the Purkinje cells of the cerebellum. These filaments exist, in an identical form, in the mycelial ramifications of *Mucor*. Figure 4, Plate 47, shows one of the small ramifications A of *Mucor corymbifer*, dividing into two branches, B, C, also bearing spores, D, D, applied against the filaments.

Figure 2, plate 48, shows the ramification of a large myelinated fiber of the sciatic of rabbit A, divided into two trunks, B and C; the partitioning lines are marked at the point of division and affect the form they have in the ramification of the large mycelial filaments of *Mucor*, as shown in Figure 3, showing the division of a mycelial filament of *Mucor corymbifer.*

Figure 4, Plate 48, shows two branches, A and B, of a myelinated fiber, one of which, B, bears a series of string-like varicosities and ends in a fruiting body C.

This arrangement of string-like varicosities is found identically on mycelial filaments of the *Mucor* type. In Figure 5, Plate 49, this arrangement exists in a terminal branch of *Mucor corymbifer.*

These varicosities, which may be more pronounced, are identical to those described on Remak fibers; these, or at least a large number of them, are ramifications of myelin fibers; a myelin fiber emits ramifications without myelin, just as a large *Mucor* filament, equipped with a myelin sheath, emits bare filaments without a sheath; it follows that the presence or absence of the latter does not allow us to conclude that there is a difference in functions between myelin fibers and Remak fibers.

Figures 1, 2, 3, and 4 of Plate 49 contain various types of branching of the Mucor mycelium (1 and 3, *Mucor* form of the agent of icterohemorrhagic spirochetosis; 2, *Mucor corymbifer*; 4, *Mucor* isolated from the brain of a rabid dog). Other forms will be found in the numerous figures in this work representing *Mucor.* I point out that the forms of Figures 1 and 3 are found in the ramifications of branches emanating from cells of the sympathetic ganglia.

Figure 1, Plate 48, represents the *Mucor* form of a culture of bovine tuberculosis; it has been reproduced to show a mycelial tube A, presenting transverse striations; a mycelial tube B, an interannular constriction D, and a spore C which represents a Schwann nucleus.

Figs. 1, 2, 3, of Plate 50, are reproduced to show how a small-caliber mycelial filament A, Figure 3, comes to form a large bundle of small-caliber filaments. These three photographs represent the same filament AB, of the mucor form of a bovine tuberculosis culture, continuing into BC, branching out, then CD, and forming the large bundle in Figure 1. By examining these mycelial filaments, the axis cylinder and the external sheath of some of them can be distinguished.

11. Branching of the axis cylinder within the myelin sheath and constitution of this sheath.

As in connective fibers, the axis cylinder of myelinated fibers divides into branches which remain grouped in a bundle and give it the fibrillar structure that is known. Fig. 3, plate 51, represents a rabbit myelinated fiber emitting its terminal ramifications. Within the myelinated sheath, the nerve fiber divides in the same way.

Figures 1 and 2, plate 51, show the same fiber A, divided into fibrils and presenting an interannular constriction B, at the level of which the fibrils join together in a tight bundle reconstituting the cylinder-axis. Fig. 2 shows other similar fibers, notably fiber D with its division into fibrils at F and a constriction at E.

Subsequently, these fibers lose their sheath and only a bundle of bare fibrils remains, several of which, II, H, are visible in Figure 2; nothing distinguishes them from bundles of connective fibrils, being like them difficult to stain with basic colors. These different arrangements exist identi- cally in in vitro cultures of molds. Figure 4 shows a mycelial tube of *Mucor* A (isolated from the brain of a rabid dog) dividing to form a bundle of fibrils.

Plates 50 and 52 show with the greatest clarity the ramification of the cylinder-axis into fibrils in the mycelial filaments of *Mucor.* Figures 6, plate 50, and 1, plate 52, are detached from the same photograph, a portion of which has been enlarged in Figure 5, plate 50 (gr. 750 D) and in Figure 2, plate 52 (gr. 500 D).

In Figure 2, plate 52, the division of the cylinder-axis into fibrils is visible on almost all the mycelial filaments which run through it.

The cylinder-axis of filament A (Figs. 1 and 2, pl. 52) is made up of a group of fibrils which, like it, ramify during their journey; it presents a series of ovoid, string-like swellings, C, C, already noted previously. These swellings appear to correspond to the main branching points or to the emission points of the largest branches; they exist in an identical form in myelinated nerve fibers.

Figure 5, plate 51, shows a bundle of myelinated fibers from the rabbit, several of which present these string-like swellings A, A, A.

Figure 2, plate 52, shows two aged mycelial filaments D, E, presenting the longitudinal striation of the membrane which has been described on myelinated fibers. Fig. 5, plate 51, also shows aged mycelial filaments from an undetermined *Mucor.*

A comparative examination of a mycelial filament of *Mucor* (D, D, fig. 5, pl. 50) and a myelinated fiber from the rabbit sciatic nerve (D, D, fig. 4, pl. 50) reveals the constitution of the myelinated nerve fiber; the axis-cylinder emits a multitude of branches perpendicular to its axis, thin and short, very ramified, each ramification of which carries a fine round spore. The Schwann sheath is made up of other branches of the axis-cylinder, noted above, arising at an acute angle and forming the external membrane by their ramifications covered with appendages and spread out *on a single plane.*

The small perpendicular branches, described above, push their ramifications as far as the Schwann sheath, with which they become entangled and to the formation of which

they contribute by constituting at the same time a supporting carcass for the fiber. Thus at the same time forming a supporting carcass for the fiber. Thus, between the axis-cylinder and the Schwann sheath, formed by its long, oblique ramifications, there is a space in which the fine perpendicular branches **(1)** ramify and spread out.

Summarizing these observations, we see that the myelinated nerve fibers are identified, down to their smallest structural details, with mycelial filaments of *Mucor*.

Remak fibers, which ramify like connective fibers, are also mycelial filaments of molds. By carefully searching a large nerve trunk like the sciatic, by analyzing all the elements found there, one cannot find a single element that cannot be identified as a mold element. The sciatic is entirely composed of:

1. Myelinated fibers.
2. Remak fibers.
3. Connective tissue elements.
4. Other isolated elements, germinative masses of various sizes and spores of different dimensions.

Now, all these elements are made up of mold vegetation; there is no doubt about this. We must therefore conclude:

1. That these mold elements, which are plant-like, fulfill the role of nerve conductors and connect the terminal organs to the nerve centers.

2. Or that these elements are parasitic and play no role in the phenomena of nerve conduction.

I investigated whether other elements could exist in the nerves and play the role of conductors. I thought that this role could be fulfilled by the lamellar sheath and the sheath of Henle**,** which merges with the sarcolemma of the muscle fibers. However, this sheath is itself made up of fine ramifications of the mycelial fibers that have just been described.

Is there an unknown substance between the nerve fibers that fulfills the role of conductor? I have not been able to detect its presence. Thus, the nerve is of the same nature as connective tissue; both are made up of mold elements.

The results acquired so far imply that, if the myelinated nerve fiber is a mycelial filament of *Mucor,* the nerve cell of the centers from which it emanates must also be a cell or organism of *Mucor.*

(1) *These fine perpendicular branches constitute the bodies which, seen partially in transverse sections, were called by Nageotte "mitochondria of the myelinated sheath."*

If it is demonstrated that this cell is of another nature, it will follow that the myelinated fiber cannot be a nerve fiber. The observation therefore leads us to a new, inevitable study of the fundamental elements of nerve centers and their nature.

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III. Study of the fundamental anatomical elements of nerve centers.

The aim of this study was to determine whether, among the various anatomical elements contained in a fragment of grey or white matter from the nerve centers, there are any that do not possess the characteristics of mold elements. From this point of view, I examined the grey and white matter of the spinal cord of the cerebral hemispheres and the cerebellum.

Study of the anatomical elements of the spinal cord.

The observations were made on preparations obtained in the following way: A small piece of the grey or white matter of the cord is placed and crushed between two glass slides, which are then slid over each other to separate them.

It is allowed to dry, stained with dahlia, and then washed in warm water to remove excess dye. In these preparations, we find nerve cells affecting, in the spinal cord of the ox, the different forms photographed in plates 53 (Fig. 1 to 8) and 54 (Fig. 3 and 4).

Examination of these cells under high magnification showed that they are mold perithecia; like these, they are formed by the crossing of several mycelial filaments emitting branches which form, through their ramifications, a very fine areolar tissue. In the center of the perithecia, these filaments coil into a ball to form a spherical mass or ovoid in which large spores form. This central mass is what histologists have called the cell nucleus (Figs. 1, 2, 5, 6, 8, plate 53). To understand this constitution, the reader should examine the figures with a magnifying glass.

A first examination of the cells represented in plate 53 shows that they have very varied, one might even say random, shapes; we realize that no law governs these shapes/forms. This is also what we notice in the perithecia of molds cultivated in vitro. *Their form is arbitrary because it is determined only by the chance of the meeting of the mycelial filaments that constitute them.* It is for this reason that certain nerve cells of the spinal cord take on such bizarre forms (Fig. 4, pl. 53; fig. 4, pl. 54). By examining under a magnifying glass figures 2, 6, 7, 8, plate 53 and 4 plate 54, we note:

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1. That the mycelial filaments forming the perithecia divide, upon entering it, into filaments which branch out in turn and bear fine stalked spores (Fig. 2).

2. That the central core is formed of large granules (Fig. 2) identical to those scattered in figures 6 and 8.

3. That stalked spores, identical to these granules, are still adherent to the mycelial filaments (Fig. 3, 7, pl. 53; fig. 4, pl. 54) which give rise to them.

4. That certain mycelial filaments bearing spores enter into the constitution of multipolar cells (Fig. 7, pl. 53).

Figure 3, plate 54, represents a large mycelial trunk A, detached from its connections and emitting numerous lateral branches; it is surely a mycelial trunk, because we notice the formation of spores by the branches it emits. Now, three of the branches which arise on this mycelial trunk constitute, almost immediately after their birth, a characteristic multipolar cell A. Plate 55 represents another single mycelial trunk of considerable dimensions, emitting numerous branches whose ramifications can be seen spread out. By carefully examining, with a magnifying glass, all parts of the figure, we find the attributes of mycelial vegetation. We also note that at certain points multipolar cells of different sizes have formed. These are therefore perithecia formed by the large mycelial trunk for the production of spores.

These different observations allow us to affirm that the multipolar cells of the spinal cord are made up of tangled mycelial filaments; they are therefore perithecia of a mold.

New observations, made on a transverse section of the cat's spinal cord impregnated with silver nitrate, will allow us to verify this conclusion.

Plates 56 and 57 contain photographs of the regions of the cord studied. We will find there the nerve cells whose photographs are reproduced in plates 58 and 59.

A nerve cell of characteristic shape, all of whose elements, very clear and distinct, can be studied with a magnifying glass, is contained in Figure 5, Plate 58.

Six nerve fibers, each consisting of a bundle of elementary fibers, constitute it by the entanglement of these. These elementary fibers are mycelial filaments, because, examined with a magnifying glass, they are seen to emit along their path short appendages, spiral or not, ending in a fine round spore.

A number of these filaments coil to form the central mycelial coil, considered the nucleus of the nerve cell. In reality, the circular coiling of the filaments exists only at the periphery of the coil, forming a sort of shell enveloping the central part; this is made up of wavy fibers emanating from the filaments of the shell, twisted in all directions, entangled, and bearing fine spores. This constitution of the central part is visible in a young cell placed in the center of Figure 3, Plate 58.

In older cells, the central part of the nucleus has been transformed; the mycelial filaments have largely disappeared and are replaced by large oval basophilic spores (A, Fig. 5, Pl. 58) about 5 µm long by 2 ½ to 3 µm wide. This entire constitution of the nucleus is clearly visible in Figures 3, 4, 5, Plate 58, and 2, 3, 4, Plate 59; Figures 2 and 3, Plate 59, show this constitution with as much clarity as the cell which has just been described; in Figures 3, Plate 59, and 4, Plate 58, in particular, we can distinguish the path of the elementary fibers of the nerve fibers which form the cell and we see them reach the shell to constitute it by winding.

The elementary fibers that do not enter into the constitution of the shell emit branches whose fine and multiple ramifications are covered with small appendages, spiral or not, all ending in a fine spherical spore, and constituting a fine areolar tissue (Fig. 1 and 2, pl. 59) occupying all the space of the cell around the nucleus.

The protoplasm of the nerve cell (like that of any cell in general) is therefore constituted by this fine areolar tissue of mycelial nature and by the aqueous substance contained in its meshes.

The mitochondria, elements that have been described in very variable forms, are the fine spores that cover the mycelial filaments constituting the areolar tissue, the appendages that carry them, or the fine mycelial ramifications themselves, seen only, in sections, over a short portion of their path.

Thus, sticking strictly to the facts, the multipolar and even bipolar nerve cells (Fig. 4, pl. 59), shown in plates 58 and 59, are constituted by a tangle of mycelial filaments, forming a spherical or ovoid mycelial shell in which spores are born *and whose purpose is the formation of these spores. They are therefore perithecia of molds.*

Further confirmation will be provided later by the presentation of the forms of perithecia in molds and the reproduction, in these, of the characteristic forms and constitution of the multipolar nerve cells of the nerve centers.

The constitution and nature of these fundamental elements having been established, it remains to determine the nature of the other elements observed in the medulla, fibers, agglomeration masses, granulations, etc.

Previously, we have already examined the large trunks and nerve fibers; along their path we find mycelial dilatations, segmentation marks, and other elements characteristic of molds: spores of various sizes.

In the white substance prepared by crushing, we observe these various elements: certain filaments end in large spores or spherical fructifications 6 to 8 µ in diameter (Fig. 3, pl. 60); identical fructifications are noted (Fig. 3, pl. 60) in a mold obtained by cultu-

ring the brain substance of a rabid dog **(1)**. Finally, when we follow the nerve fibers or the branches they emit, we see that they divide at their ends into fine branches ending in spirals and covered along their path with small spiral spore-bearing appendages or short-pedunculated spores (A, fig. 1 and 2, pl. 60). These spiral branches and appendages form, by their entanglement, an areolar tissue which fills the spaces between the fibers and the nerve cells.

It is these fine spiral filaments and their spores (zymospores) that constitute the lecithin extracted from the nerve centers. In reality, a mixture of various bodies is extracted, with which the lecithin is mixed.

In addition, in the crushed preparations, agglomerated masses formed by the spores described above are observed; some emit mycelial filaments that specify their nature (Fig. 1, pl. 73).

Are there other elements in the spinal cord that do not exhibit mold attributes? On crushed preparations, I have not found any; on the other hand, by examining thin sections of marrow, impregnated with silver nitrate, numerous microscopic fields such as

those whose photographs are included in Figures 1 and 2 of Plate 54, we do not find any more. In any sufficiently studied element, we find the connection with the mold from which it emanates.

From these observations, it follows that the spinal cord contains only mold elements, elements in various states attesting to their evolution.

Faced with such a result, it was necessary to investigate whether, in molds in vitro, elements identical to multipolar cells are found.

I photographed the various forms of the perithecia of a common, non-fructified mold, collected from the hair of a patient suffering from microsporia and cultivated on agar.

These forms are contained in plates 61, 62, and 63. We note in these plates:

1. That the forms of mold perithecia are identical to those of the multipolar cells of the spinal cord.

2. That these forms have nothing characteristic and result, like those of multipolar cells, from the chance encounter of the mycelial filaments that give rise to them.

3. That their size is very variable, like that of the multipolar cells. By comparatively examining the perithecia A, fig. 1, plate 61, A, figs. 2, 3, 4, plate 62 and the multipolar cells contained in plates 56 and 57, no essential difference is found.

(1) *This mold was recognized as a gonidial form of the organic mold of carrots, and the pathogenic agent of rabies.*

Certain multipolar cells of the pith have a spherical shape (Fig. 5, pl. 59). In the mold in question here, numerous perithecia of the same shape existed (Figs. 2, 5, 6, 7, 9, pl. 63); these figures show that the perithecia is frequently formed by the winding of one or more filaments (Fig. 4, 5); one of these perithecia (Fig. 1, pl. 63) is observed at the very moment of its formation. Another (Fig. 6) shows in the lighter central part the areolar tissue and several spores, including a large oval one, formed in this tissue.

Figure 5, plate 62, contains a photograph of a perithecia of the malaria mold, cultivated in vitro. This perithecia, formed by four filaments, A, E, F, G, has been disorganized by the preparation maneuvers; by examining it with a magnifying glass, we find: the shell

of the nucleus formed by winding filaments, the large round spores (K) contained in this shell; the areolar tissue surrounding the shell; the numerous very small spore-bearing appendages, spiral or not, which cover the filaments forming this tissue.

These observations prove that, in in vitro mold cultures, there are formations (perithecia) identical to the multipolar cells of the nerve centers.

The observed facts therefore necessarily imply the following conclusion, however inadmissible it may seem:

All the anatomical elements of the spinal cord, nerve fibers and cells, are elements of molds growing in this organ, and it does not contain any figurative elements of another nature.

If the spinal cord is indeed of this nature, one should, by spreading the pulp of this organ on artificial culture media, obtain the gonidial forms of the molds growing there. Experiments verify this fact. By cultivating the pulp of the substance of the brain or the spinal cord, one obtains these varied gonidial forms belonging to a single species of mold.

Moreover, by examining some of these forms, one finds perithecia similar to the multipolar cells of the nerve centers.

Plate 64 contains a series of photographs of a mold obtained by culturing the grey matter of the hemispheres of a stillborn child. Examination of the perithecia under a magnifying glass will show their identity with the multipolar cells of the centers.

These observations, which agreed with previous ones made on the nerves and connective tissue, constituted a troubling enigma whose solution required new observations on the anatomical elements of the brain.

Study of the anatomical elements of the cerebral hemispheres.

Examination of the figures representing the various anatomical elements of the brain in histology treatises alone allows us to conclude that they are mold elements. There are few figures in Ramon y Cajal's *Treatise on Histology*, for example, that do not allow this identification.

I cite only a few examples taken from different collections:

The pyramidal cells of the cat's cerebral cortex, represented in figures 359, 364 of Ramon y Cajal's *Treatise on Histology*, are elements of molds in vegetation, recognizable by the varicosities of the dendrites (mycelial dilations, intercalary chlamydospores) and the lateral appendages carried by the mycelial filaments (Fig. 2 and 3, pl. 65). These appendages have a shape identical to the fructifications of the monosterigmate gonidian form of molds.

Figures 342, 354, 372, 374 of this same treatise show the identity of the large pyramidal cells of the cerebral cortex and the multipolar cells of the grey matter of the spinal cord; like the latter, they are mold perithecia formed by the entanglement of mycelial filaments.

Figure 184 of Prenant's *Treatise on Histology* represents a Purkinje cell of the cerebellum (Fig. 3, pl. 65); this cell is constituted by a large germinative mass giving rise to a large, abundantly ramified mycelial trunk, bearing at the end of its branches spiral or non-spiral spore-bearing appendages, characteristic of the terminal ends of the mycelial filaments of molds.

Figure 430 of Ramon y Cajal's *Treatise on Histology*, representing a section of the cerebral vesicle of a urodele, contains, in all its elements, proof of their nature; a part of this figure, enlarged (Pl. 66), clearly shows the shape of the appendages of the branches of the nerve cells; these are indeed the fructifications of the monosterigmate gonidian form. The granulations at the end of the appendages are spores; detached, they constitute the multitude of corpuscles of the same size that the nerve substance contains.

Let us now turn to the direct observation of the anatomical elements.

This was done on preparations obtained by crushing a fragment of grey or white substance between two glass slides, and then sliding the slides over each other to detach them. In such preparations of the grey substance of the hemispheres, colored by the dahlia, we observe:

1. Elements consisting of a mass of variable shape emitting highly branched filaments: these are the cells of the cerebral cortex, notably the pyramidal cells.

2. Numerous granules of 0 µm3 to 1 or 2 µm, and larger spherical bodies of variable size; the majority are 8 to 12 µm; some are ovoid.

Cells of the cerebral cortex.

All these elements have common characteristics that allow them to be easily identified: the filaments they emit bear varicosities or mycelial dilatations, flat basophilic spores closely attached to them; they ramify and some of their branches bear at their ends spores or fruiting bodies of various sizes; they carry along their path short, straight or spiral spore-bearing appendages, ending in a small round spore.

Let us take as an example the pyramidal cell of the sheep's cerebral cortex contained in figure 1, plate 67. The body of this cell is located at the bottom of the figure, it is easily found by following filament B with a magnifying glass; this, abundantly branched, emits branches which carry: either round or oval fruiting bodies of 8 to 12 µm at their end; or smaller spores of about 1 or 2 µm; or spores of 0 µm. 3 to 0 µm 6 carried at the end of the spiral appendages of the fine ramifications.

It is also noted, by following the mycelial filaments, that the varicosities or dilations are often real, but also that the appearance of varicosities is given by the presence of flattened, oval, basophilic external spores, born on the filaments and closely attached to them; they are pedunculated and, if the coloration is sufficient, one can follow this pedicle to its birth. It has been shown, previously, that the mycelial filaments of mucor emit branches presenting the same appearance, due to spores of the same shape attached to them (Fig. 4, pl. 47).

Pyramidal cells are therefore germinative masses of mycelium giving rise to mycelial arborizations which constitute the cell's dendrites. These dendrites do not have a simple shape as one might believe from the figures given in histology treatises; their ends are divided into a multitude of fine, wavy branches, bearing spiral or non-spiral spore-bearing appendages, all ending in a fine spore, constituting by their intricacy the tissue of the grey matter.

The mycelial arborizations also bear, at the end of certain branches, pyriform formations 30 to 35 µm wide on average and 70 to 80 µm long; there are two formations of this kind placed side by side at E; they are known and described under the name of encapsulated balls (Ramon y Cajal, Nageotte); their structure will be analyzed later.

Certain pyramidal cells have a very developed vegetation; only one of the ramifications C of the broken branch A, figure 1, plate 68, is nearly a millimeter long.

From this analysis, it follows that the fundamental elements of the cerebral cortex, the pyramidal cells, belong to a mold growing in the brain. The rounded bodies of 8 to 12 µm or the smaller isolated granulations, which are innumerable in the preparations, are also easily identifiable, because a large number of them are found still bearing the arborizations which gave rise to them; their origin is as easy to recognize as the apples fallen to the ground under the apple tree which formed them.

Examination of the white matter of the hemispheres provides indications similar to those we obtained by examining the white matter of the spinal cord. All the elements can quickly be recognized as belonging to molds.

Examination of any other region leads to the same conclusion. If, for example, we examine the corpus striatum, we find large elements identical in structure to the pyramidal cells of the cortical substance. Figures 1 and 2, plate 68, represent an enormous cell of the corpus striatum; the germinative mass or body of the cell, disorganized by crushing, is located in Figure 1; the dislocated branches are scattered in Figure 2; one of them is, from one end to the other, a millimeter and a half long; the cell could therefore extend over a length of 3 millimeters, the figure containing only a part of it; we recognize there enough of the identity of vegetation with a pyramidal cell, so that it is unnecessary to describe it.

* * *

From these observations, it must be concluded that all the figurative elements contained in the cerebral hemispheres, from the largest to the finest granules, are elements of a mold growing in the brain.

Culturing the cerebral pulp of the hemispheres of different individuals of the same species on agar gives rise to diverse gonidial forms belonging to a single species, and identical to those obtained by cultivating the pulp of the spinal cord or the connective tissue.

Study of the anatomical elements of the cerebellum.

The observations will focus on the elements of the grey matter, the white matter not having provided me with indications different from those obtained in the study of the white matter of the spinal cord. As with the cerebral hemispheres, the study was carried out on crushed preparations.

Here is the nomenclature of the elements observed:

1. *Purkinje cells.* Figure 1, plate 69, represents one of these cells emanating from an elongated sclerotium of irregular shape. The large trunk emitted by this sclerotium ramifies abundantly into a certain number of branches; some, long, continue their course; others, short, divide into a large number of fine wavy filaments forming a tuft and covered with fine spiral spore-bearing appendages.

What has been stated previously for the vegetation of pyramidal cells also applies to the vegetation of Purkinje cells, which are identified using the same characteristics. Besides the difference in their shape, Purkinje cells are distinguished from the pyramidal cells of the cerebral cortex by the fact that they do not bear rounded, basophilic fruiting bodies of 8 to 12 µm; they bear only round, basophilic spores of 4 to 6 µm.

This remark, which applies to the various elements of the cerebellum, clearly differentiates them from those of the cerebral hemispheres. Figures 1 and 2, plate 70, contain photo- graphs of mycelial branches of the elements of the cerebellum, on which the varicosities can be studied with certainty under a magnifying glass. We will see that, often, the varicosity is determined by the adhesion to the filament of a flattened spore emerging on its path. Indeed, spores of the same shape exist isolated at the bottom of the figure, and others, slightly detached from the filament, are still adherent to it by their pedicle.

2. Numerous fragments of the vegetation of other categories of cells of the cerebellum, various forms of the elements observed are represented in figs. 2 and 3, plate 69; figs. 1, 2, 3, 4, plate 70, and fig. 1, plate 71.

These are, for the most part, parts of the mycelial vegetation detached from the original sclerotium; this vegetation extends far from this sclerotium, at least 1 or 2 millimeters, as shown in figs. 1, plate 71, and 2, 3, plate 69, this independently of long fibers that it can send to the medulla oblongata for example. Certain elements (Fig. 4, pl. 70) are constituted by an elongated sclerotium, irregular in shape, emitting large mycelial trunks; these elements cannot be seen in thin sections of the organ, being generally sectioned in a direction oblique to their longitudinal axis. The mycelial elements they emit easily identify them.

3. A very remarkable element, but one of which there are very few samples in the preparations, is represented in Figure 3, Plate 70. It is constituted by a round or oval, basophilic germinative mass or sclerotium, emitting a large number of very long, straight, basophilic mycelial filaments, most of them unbranched, and other smaller, branched filaments, bearing spiral spore-bearing appendages at their ends.

4. Encapsulated balls. In most elements of the cerebellum, the mycelial vegetation bears numerous pyriform formations approximately 70 µm long by 30 to 35 µm wide

(Fig. 1, Plate 71). These formations, already described under the name of encapsulated balls (Ramon y Cajal, Nageotte), arise at the end of a single filament and are formed by its division into fine, flexuous branches (A, B, fig. 2, pl. 71) which curve to give the element its pear shape (Fig. 1 and 2, pl. 72).

These branches and their own ramifications bear spore-bearing appendages and constitute an areolar tissue in the middle of which is formed a large single spore: the ball; this, strongly basophilic, 12 μ in diameter, results from the fusion of the basophilic spores formed on the mycelial filaments which surround it. This is the mode of formation of the perithecia and their spores (Fruchtkörper).

It is curious to note that this large spore is precisely the size of the spores so numerous in the brain and which at first seemed to me to be born directly at end of the mycelial filaments without passing through the pear-shaped formation; if we examine Figure 1, Plate 67 (grey matter of the cerebral hemispheres) under a magnifying glass, we see, however, that certain spores of 12 μ in formation are not very colored at the beginning and contain in their center a small spore of 3 to 4 μ; they therefore have at this moment the type of small encapsulated balls of 12 μ and the central spore comes to occupy all the space of the formation. There is therefore, in the cortical grey matter of the hemispheres, an infinity of these small encapsulated balls of 12 μ which become totally basophilic; I was able to observe them in perfect condition on the mycelial filaments of the pyramidal cells of the cerebral cortex of the rabbit (A, fig. 2, pl. 67).

Does the central basophilic mass of the large encapsulated balls of the cerebellum increase in volume and end up occupying the entire space of the formation to become a germinative mass or sclerotium, the starting point of a new vegetation? This hypothesis is made very probable by the following fact: apart from the main spore of 12 μm that the encapsulated balls contain, they form other spores, some of them quite large (3 to 5 μm, Figures 2, 3, 5, pl. 72) and, on the other hand, certain encapsulated balls become so strongly basophilic in their entirety that their contents are no longer clearly distinguishable (Fig. 3, pl. 72).

5. In addition to the encapsulated balls, the same element can give rise to spherical perithecia (Fig. 2, plate 71, and Fig. 6, plate 73) carried at the end of a mycelial filament of fairly large diameter and formed by the winding of this peduncle; one of these perithecia is contained in plate 71 near the right margin of Figure 2; another, contained in Figure 6, plate 73, is formed by the winding, quite visible under a magnifying glass, of the filament which constitutes it. These coiled filaments form spores which germinate in the very interior of the perithecia and give it the echinulated appearance when the mycelial filaments begin to appear on the outside; several of these perithecia,

detached, exist in figures 4 and 5, plate 73. One of them (Fig. 5) is in the process of formation; these perithecia therefore become germinating masses or sclerotia.

The mode of formation of these perithecia, studied in molds cultivated in vitro, is represented in figures 1 to 5, plate 63; another, completely formed, is represented in fig. 8, plate 73. These perithecia, spherical in shape, can be grouped in a single layer of cells; these are the elements that Ramon y Cajal called glomeruli (B, fig. 430, Ramon y Cajal, *Histology of the Nervous System)*.

6. Figure 3, plate 73, contains a group of germinating spores, each constituting a neuroglial cell in formation.

Analysis of these elements is sufficient to identify them. It is nevertheless useful to show that, in the organism, mold elements *vegetate in a form that does not differ from those they affect in in vitro cultures on artificial media.* Mycelial fibers, such as the one shown in Figure 2, Plate 74, are not rare in in vitro mold cultures; along their entire course, we find the very clear transverse marks of mycelial partitioning.

Finally, in other preparations, the observer has before his eyes the most characteristic mycelial filaments of *Mucor*. Figure 1, plate 74, shows a large nerve fiber A of the grey matter of the sheep's cerebellum, 20 µm wide; it is made up of a bundle of secondary fibers; the constriction it carries is due to the torsion on its axis of the upper part of the fiber; but it emits a filament B carrying on its path dilations of which one C in the shape of a top is characteristic; we will see two identical dilations placed on the path of filament B (Fig. 3, plate 34) emanating from a large mycelial filament of *Mucor* obtained by culture of the cholera vibrio (Bologna vibrio. Pasteur Institute).

Numerous other examples will be found in Plate 25 and another in Fig. 1, Plate 39 (*Mucor* form of human tuberculosis or human organic mold).

A large mycelial trunk of the Mucor type contained in Figure 3, Plate 34, divides at its upper part into a certain number of branches which have a shape identical to that of the filaments of the dendrites of the pyramidal cells of the hemispheres, to the branches of the Purkinje cells, etc.

Finally, it will be seen, by examining Fig. 3, Plate 74 under a magnifying glass, that, in the cerebellum, the 5 to 6 µm spores are born, without any possible doubt, at the end of mycelial filaments arising on others which bear spiral spore-bearing appendages.

The fine spores borne on the spiral appendages constitute the *zymospores* or *ferments* of the nervous tissue. Water, containing a portion of crushed brain tissue, being examined at high magnification between slide and coverslip, we see that these zymospores, formed by the spiral appendages, possess Brownian motion.

Wanting to push the identification of all the forms observed even further, I photographed and exhibited:

1. In plate 73 (Fig. 2), a *Mucor* fruiting body whose spores germinate inside the capsule (identity with the perithecia of figures 5 and 6).

2. In figure 9, plate 73, encapsulated balls growing on the mold obtained by culturing various rat tissues.

3. In Figure 6, Plate 72, encapsulated balls formed by mold obtained by culturing uterine cancer.

4. In Figure 7, Plate 72, a mold obtained by culture of a fragment of tuberculous human lung tissue; it shows the formation of spores identical to those we observed in the connective tissue of the nerves and in the nerve centers. It forms a perithecia by winding a mycelial filament.

We are now in possession of sufficient elements to make a judgment.

In the most essential organs of an animal, nerves, spinal cord, cerebral hemispheres, cerebellum, all the elements represented are constituted by the vegetation of a mold.

This point is demonstrated with such multiple and convincing evidence that no doubt can be preserved; the accuracy of the fact is verified by *definitive material proof:* the very easy obtaining of the mold which constitutes the anatomical elements of the nerve centers by inoculating a culture medium with the cerebral pulp.

But, if the nature of the anatomical elements that constitute the nervous system is established with certainty, no fact directly demonstrates that it is indeed these anatomical elements that fulfill the nervous functions; could these functions not be fulfilled by an unorganized substance filling the spaces between the formed elements? Current physiological knowledge, the relationships that exist between the degeneration of the elements and the loss of nervous functions, make such a hypothesis very improbable.

It was, however, necessary to establish that there exists a cause-and-effect relationship between the elements of molds and the physiological phenomena of which the nerve centers or other organs are the seat.

I thought I could obtain this proof by studying muscles. These organs are said to contain a contractile albuminoid substance, myosin, which affects a very special physical disposition in muscle fibers; a very special organ for transmitting excitations controls their activity: it is the arborization or terminal motor plate of nerve fibers.

I therefore began this study with a view to investigating whether the molds, whose presence I had observed in nerve centers and nerves, play a role in muscle contraction.

IV. Study of the Terminal Motor Endplates.

My observations focused on the nerve endings in the muscles of the snake and those of mammals; they were made on preparations obtained either by crushing between two blades, or by dissociation, or by dissociation in salt water followed by crushing. It is essential, for this study, to comparatively examine preparations stained with basic colors and others impregnated with silver nitrate or gold.

Terminal motor endplates of the snake muscles.

We will examine these arborizations in a preparation impregnated with gold; their shape is known; it is only a matter here of specifying their constitution and determining their nature. Plate 75 shows two of these arborizations photographed at a magnification of 610 diameters, on the left, and 1100 diameters, on the right.

The nerve fiber descends obliquely on the arborization and divides into several branches, in Figure 1, before reaching it; the motor endplate must therefore include, here, several secondary terminal arborizations. In Figure 3, the nerve fiber branches immediately into a single terminal arborization.

The constitution of the arborization can be studied with sufficient clarity in figures 3 and 4; to constitute it, the nerve fiber divides or spreads into a certain number of branches, 5 or 6 or more, constituted either by a series of large articles placed end to end, or by a thin filament carrying, after a certain path, a series of oval masses placed end to end; these articles or masses emit in their turn: either lateral ramifications, or short simple or bifurcated appendages, carrying at their end one or two spherical masses having the appearance of spores of 4 to 6 μ. These articles carry, in addition, finer appendages ending in fine spores (0 μ. 5 to 0 μ 6) (zymospores). The terminal arborization, formed by a mycelial filament of mold, therefore also presents the characteristics of a mycelial arborization.

I investigated whether there are closer relationships between the terminal arborization and the underlying elementary muscle fibers.

There appears to be a direct relationship between certain dilated articles of the motor endplate and the elementary fibers constituting the primary fiber contained in the sarcolemma; we notice, scattered in it, along its length and between the elementary fibers, oval masses which, obviously, play the role of germinative masses; they emit filaments

which, perhaps, will constitute elementary fibers. It also seems that certain oval articles of the arborization emit a filament which will insinuate itself into the mass of parallel elementary fibers. These are facts of very delicate observation and which can only be determined with certainty by clear and sharp photographs, very difficult to obtain.

Terminal motor plates of mammals.

Following Ranvier's advice, I turned to the intercostal muscles of the rabbit for this study; small muscle bundles are dry dissociated or crushed on a glass slide on which a silver nitrate solution is deposited. They are exposed to the light of a 50-candle- power electric lamp for one or more hours depending on the desired result; they are then washed and treated with a developing solution of metol and hydroquinone. They are washed one last time with distilled water and mounted in Canada balsam.

The observations I have made on such preparations do not agree with those of Ranvier**.** This author described, as terminal plates, granular eminences in which the nerve fibers terminate.

I observed these eminences, white plates not impregnated with silver nitrate, which have either the shape of a sole A, B, fig. 1, plate 76, or more often an irregular shape C, fig. 1, plate 76; A, B, C, fig. 2, pl. 76; A, B, C, fig. 2, plate 77; A, fig. 3, plate 77. These eminences appear to be degenerate arborizations.

If we examine those which have an irregular shape, we see that, often, they have the shape of normal arborizations at certain points of their periphery (A, fig. 3, pl. 77). On the other hand, we find normal arborizations of the same shapes (A, fig. 4, pl. 77) which appear to be in the process of evolution and which are impregnated with silver nitrate.

My observations differ significantly from the description given by Ranvier; I will set them out without further discussion, the facts that I report being easily verifiable and being justified by photographic documents. Here they are:

1. I did not observe the isolated arborizations that have been reported on the muscle fibers. I saw, on the sarcolemma of the same fiber, a large quantity of very similar arborizations, clearly detached; figures 2 and 5 of plate 77 show two different fields of the sarcolemma of the same fiber of an intercostal muscle of the rabbit.

2. The arborizations have a variable form indicating that they are undergoing an evolution. We observe clear, well-developed arborizations (Fig. 2 and 5, pl. 77), grouped in

patches, and, at other points, condensed masses of irregular shape (Fig. 4, pl. 77) indicating, by the examination of some of them (those at the top of the figure), that each mass results from the agglomeration of the elements of an arborization. This evolution of the arborization is visible in figure 2, plate 77, containing a well-formed arborization D, above which exists another in the process of condensing its elements. I believe that the white, unimpregnated masses, such as A, B, C, in the same figure, are masses of this nature whose *evolution is completed* by the total emigration of the basophilic protoplasm.

3. The distribution of arborizations on the sarcolemma is extremely variable, as can be judged by Figure 1, Plate 76. The sarcolemma possesses, in certain regions, a very clear circular striation which will be described later; in others this striation is lacking. Arborizations appear to be rare in the striated regions of the sarcolemma and numerous in the points where the striation is lacking or not very marked.

4. At the points where a nerve fiber passes, whether its direction is longitudinal or transverse, it gives rise to numerous fairly long, sinuous, varicose branches, emitting lateral appendages bearing large spores similar to those of the arborizations (fiber C, D, Fig. 2, Plate 76).

5. One of the essential points in the study of muscle fibers is *to follow the path of the nerve fibers and determine where they go.* This observation is very difficult to make because most often the fibers are broken, short and it is impossible to decide whether they emanate from a myelinated fiber or a connective filament; what in- creases the difficulty is the identity of appearance between Remak fibers and connective fibers and the fact that myelinated fibers often end in long filaments without myelin or emit Remak fibers by branching.

By following nerve filaments or such preconceptions in this way, we see that they penetrate through the sarcolemma and that, from this point, we can no longer follow them;

or that they travel for a long time on the sarcolemma in a direction parallel to the axis of the fiber and *emit laterally,* to the right and to the left, a series of regularly spaced filaments which, by dividing, constitute the membrane or determine its striation. These filaments bear numerous spiral sporiferous appendages which become entangled with the similar appendages of the two neighboring branches. This arrangement is very clear in figure 1, plate 77, where we see a fiber A dividing into two others which emit parallel branches forming the circular striae of the sarcolemma and bearing the appendages constituting any membrane. For certain fibers which present this arrangement, we can affirm that they are nerve fibers, because they present the varicose aspect cha-

racteristic of Remak fibers or, again, emanate from another fiber clearly presenting this varicose aspect.

Examining the sarcolemma in these various regions, we see that it is everywhere formed by this fine web of tangled spiral spore-forming appendages. It is therefore not an amorphous membrane as Ranvier indicates. Like all membranes of animal and plant tissues, *without exception,* the sarcolemma is made up of the web of tangled spiral spore-forming appendages, carried by the numerous ramifications of the membrane-forming mycelial filaments.

A point of capital importance is to determine whether the forming mycelial filaments are connective filaments or the terminal filaments of myelinated nerve fibers.

It is indeed the myelinated fibers that form the sarcolemma. In fact:

A. Myelinated fibers are seen running and terminating on the sarcolemma, supplying the branches that constitute the striae. These branches also bear appendages ending in large spores similar to those of the arborizations.

B. Arborizations evolve to form a single mass which, once formed, emits sarcolem mal forming branches (Fig. 4, pl. 77). It is obviously for this reason that arborizations are found mainly in non-striated regions, that is, regions without sarcolemmal-forming branches.

When looking for the formative branch of an arborization, it is often not found. Frequently, it ceases to be visible and seems to get lost in the thickness of the sarcolemma before reaching an arborization or even a group of arborizations. For each of these, the formative filament seems to emanate from the thickness of the sarcolemma into the arborization itself.

Formerly, the formative filaments are very clear and visible (Fig. 2, pl. 76).

The sarcolemma is therefore the actual termination of the myelinated nerve fibers, branching over an enormous surface area and at a point of extreme tenuity to form a sac completely enveloping the muscle fiber.

But it does not constitute the sole termination of the myelin fibers. Some branches of these pierce the sarcolemma and end in the bundle of elementary muscle fibers, where I was unable to follow them.

The sarcolemma is therefore a membrane identical to the membrane forming the electric blade of the torpedo; both are formed by the expansion of myelin fibers.

It therefore appears that, when terminal arborization has the form it does in the snake, for example, *its purpose is the formation of basophilic nuclei which will constitute germinative masses emitting the branches constituting the sarcolemma and, probably, other branches constituting the elementary muscle fibers themselves.*

Here we are in possession of a first element; the sarcolemma is constituted by the endings of the motor nerve fiber; its elements are of a mycelial nature like those of the latter.

V. Study of the Constitution of Muscle Fibers.

My observations focused on the muscles of the snake, turtle, carp, rat, guinea pig, rabbit, and sheep, stained with dahlia or impregnated with silver nitrate, after dissociation.

Only the observations relating to the snake muscles were made on preparations impregnated with gold (to make the terminal arborizations visible) and then stained with picrocarmine.

To shorten or simplify my presentation, I will immediately indicate the results of this study, immediately apparent on a good preparation.

1. The muscle fiber is a bundle of elementary or secondary fibers enveloped by a membrane, the sarcolemma.

2. Each secondary fiber composing the fiber bundle included in the sarcolemma is itself made up of two other fibers or fibrils, one straight and central, the other spiraling around it, leaving an empty space between the turns of the coil.

3. The muscle fiber is not made up of alternately thick and narrow discs of a contractile substance called myosin. Muscle striation is not the optical or visual manifestation of this arrangement of alternately thick and thin discs. It results from a phenomenon of interference of light rays passing through the fiber bundle included in the sarcolemma.

4. Each of the two fibrils constituting the elementary muscle fiber is a mycelial filament of mold bearing spore-forming and spirillary appendages characteristic of the fine ramifications of molds in the organism or in vitro.

5. The muscle fiber system is also found in connective fibers in all regions. If the latter do not possess contractility, it is because it is only in muscle that such a system acquires the chemical constitution of contractile substance.

Here is the demonstration of these conclusions:

1. *The muscle fiber is a bundle of fibers*. We clearly distinguish the elementary fibers constituting the muscle fibers or bundles:

In plate 80 (snake muscle).
In plate 78, figure 1 (elementary fibers of a carp muscle fiber).
In Plate 78, Figure 2 (muscle fiber of the tortoise's tail).

In Plate 78, Figure 3 (muscle fiber of the sheep).
In Plate 82, Figures 1, 5, 8 (elementary fibers, almost completely separated in a fiber of the soleus muscle of the fetal mouse).

Figures 2, 3, 5, 7 of this plate show the winding of certain elementary fibers of the bundle around all the other fibers.

2. *Structure of the elementary fibers.* I became aware of this structure, first of all, in the bundles themselves where it is often quite visible: in the muscle fiber of the snake, for example (A, Fig. 7, Pl. 80), then on isolated fibers.

When examining a preparation, one often finds a few isolated fibers emerging from a bundle whose sarcolemma is torn or fibers passing from one bundle into another.

In Figure 6 of Plate 79, on the edge of a snake muscle fiber, we find an isolated elementary fiber A, of which the central fibril A can be distinguished, around which the spiral fibril B is wound, all the turns of which are very clearly distinguished, with the gap between them. In other cases, in the carp for example, there are two spiral fibers winding separately around the central fiber and whose turns of turns alternate and remain very distinct. There may even be three spiral fibers; the more there are, the more the turns of turns are oblique to the central fiber.

The elementary muscle fiber therefore has the same structure as a spiral spring wound around a central axis that serves as its guide. The same device can be seen in Figures 1, 2, and 3 (Pl. 79), representing the same elementary fiber photographed three times while changing the focus of the preparation; in Figures 4 and 5, another fiber is photographed twice, also changing the focus. It even appears, upon examining Figures 1 and 2 with a magnifying glass, that the central fiber is not simple and is composed of a bundle of fine fibers that are themselves wavy and spiral, or that the central fiber emits branches that run parallel to it.

Figure 7, Plate 80, provides clear proof that the winding of the spiral fiber is not an optical illusion and is indeed real. It will be seen, under a magnifying glass, first, that the spiral fibers of the two neighboring elementary fibers A and C have a discordant winding, whereas it would be symmetrical if the spiral appearance resulted from optical phenomena.

It will then be observed, by following with a magnifying glass along filament A, that the winding of the spiral filament presents, at points B, B, B, B, numerous irregularities which are found identically on the connective fibers possessing the spiral winding device.

Finally, we will see that fiber C is made up of the union of two isolated fibers in the lower half of the figure, each having its own spiral fiber; after their union at C, a single spiral filament surrounds them. The spiral winding is also very apparent on three elementary fibers of a carp muscle fiber. By following these three fibers (central, leaning against the black mass) with a magnifying glass (Fig. 1, pl. 78), we will see the spiral winding so clear (at about half the height) that we can safely see that it is made up of two spiral fibers whose turns alternate.

3. *Striation of muscle fibers*. The striation of the muscle fiber, a bundle of spiral elementary fibers, therefore does not result from a special constitution of the fiber consisting of a succession of thick and thin or dark and light discs. The muscle fiber is not made up of such discs but of a bundle of elementary fibers in a *coiled spring* with a straight central axis.

Striation cannot result from a device that does not exist. It therefore has another origin. It is not of the same nature in preparations stained with blue (or with other dyes) and in unstained preparations heavily impregnated with silver nitrate.

In preparations stained with dahlia, for example, the sarcolemma shows almost no color and remains transparent; it does indeed have its own striation, but it is almost invisible. The striation seen in these preparations is not objective. It is due to light interference phenomena.

In preparations heavily impregnated with silver nitrate, the sarcolemma becomes opaque and bears an objective striation, formed by mycelial filaments, which has been described previously.

In preparations stained with blue or red colors, the nature of the striation appears immediately if we compare photographs taken after focusing on the upper part of the muscle fiber (Pl. 80, fig. 1 (gr. 245); fig. 3 (gr. 1100); fig. 4 (gr. 1100); fig. 5, fiber C (gr. 610)), or after focusing on the central part (Pl. 80, fig. 2 (gr. 610); fig. 5 (gr. 610)).

When the focus is on the upper part, the striation bands are straight and parallel. When the focus is gradually lowered to the center, the striation bands first break; there is even discordance between the various elements of the broken band, which are no longer juxtaposed (Fiber A, fig. 2, pl. 80) end to end and move; then, other striation

bands appear, which intersect with the broken parts of the first ones (Fiber A, fig. 6, pl. 80).

In figure 4, plate 81, these two adjustments were made automatically in fibers A and B located at two different places.

The striated bands therefore do not have their origin in the vision of a real object. They are due to optical phenomena; they are phenomena of interference of light due to the fact that the light rays, while crossing the bundle of elementary fibers, sometimes encounter the spiral fiber, sometimes the void between two fibers, and to the fact that the spiral fiber is *a round or cylindrical filament which has a transverse and oblique direction relative to the longitudinal axis of the bundle of elementary fibers,* and which, consequently, deflects the light rays in directions other than the central fiber.

Let us add that the image of striation becomes apparent because, in the path of the light rays, a membrane, the sarcolemma, is interposed, producing the same effect as a frosted glass slide, due to its constitution described above.

Thus, after demonstrating the anatomical structure of the muscle fiber, these observations show us that the striated bands do not have their origin in the vision of a real object, that is, a succession of alternately dark and light discs made up of two different substances.

The muscle fiber is therefore a bundle of coil springs; indeed, the muscle possesses, once stretched, a perfect elasticity corresponding to that of a metal coil spring, a fact established by the research of A. Chauveau**.**

We now have new notions that allow us to understand the mechanism of muscular contraction: the sarcolemma that envelops the bundle of elementary fibrils is made up of a portion of the myelin fibers of which it constitutes the termination and expansion over a considerable surface area; it is consequently the homologue of an elementary blade of the torpedo's electrical organ, and functions like it by modifying the electrical potential of the membrane, a cylindrical sheath enveloping the bundle of elementary fibers.

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These are each made up of a straight longitudinal fiber wrapped in a spiral fiber. The muscle fiber or bundle of elementary fibers is therefore a bundle of solenoids surrounded by an envelope with variable potential. The spacing of the turns of the spiral fiber of each elementary fiber is determined by a certain potential value. When this value changes, the spacing of the turns changes.

4. ***Each of the two fibrils constituting the elementary muscle fiber is a mold mycelial filament.*** This nature of the two elementary fibrils is established by the spirillary

and sporiferous appendages that they bear. These appendages are easily visible on the edge of most of the snake's muscle fibers; in isolated fibers, they are clearly seen in great number under a magnifying glass on the spiral filament, between the turns of the coil, as well as the fine refracting spore that terminates them (Fig. 1, 2, 3, 4, 5, pl. 79; fig. 1 and 2, pl. 81).

5. ***The structure of the muscle fiber is found in the connective tissue*****.** I have inserted into plate 31 (intermuscular lamellar connective tissue of beef) photographs showing fibers of various sizes consisting of a spiral filament wound around a fiber or bundle of connective fibers (Figs. 1, 2, and 3, plate 31).

Large connective bundles can themselves bear this spiral winding (Figs. 4 and 5, plate 31). I have encountered this mode of spiral winding in almost all connective tissues. A large number of connective filaments of the pima mater possess this winding, which even appears to be the rule in certain areas of preparations. But it is in intermuscular lamellar tissue that I have encountered this system in its most developed form.

* * *

The nature and structure of muscle fibers being determined, I sought to understand their mode of formation.

In the body, every element of a tissue (with the exception of spores, zymospores, leukocytes, blood cells) ***is connected to a mycelial filament or branch which, itself, emanates from a sclerotium. The elements of muscle cannot escape this law.***

I sought to determine whether muscle fibers are autonomous or whether they result from the transformation of connective fibers or even myelinated fibers.

To this end, I studied the muscle fibers of guinea pig fetuses approximately two-thirds of the way through gestation.

We will examine successively, in these muscles:

1. The constitution of primary muscle fibers (bundles of secondary fibers) and secondary fibers.

2. The elements contained in the muscles of the fetus, independent of the muscle fibers: fruiting bodies, sclerotia, mycelial fibers.

3. The origin or mode of formation of primary muscle fibers and secondary fibers.

1. Constitution of the primary muscle fibers (bundles of secondary fibers) and secondary fibers of the fetus.

We will limit ourselves here to noting that the muscular fibers have the constitution which was established previously. In Figure 5, plate 82, an intact muscle fiber in A, slightly dissociated in B, shows with great clarity all the secondary fibers which constitute it; these are distinguished with just as much clarity in the more completely dissociated fiber of Figure 1.

In other figures we find a new element: The secondary fibers are held in bundle by spiral fibers (Fig. 2, 3, 6, 7, pl. 82). The winding is carried out by three spiral fibers whose turns follow each other regularly; if we numbered the turns of turns on the fiber, we would in fact see that turns 1, 4, 7, 10 are made up of the same spiral fiber and the others by the two other fibers.

One of the bundles in Figure 3, whose secondary fibers are semi-transparent, shows this device with such clarity that each spiral fiber is visible, even behind the bundle, through transparency. The primary muscle fiber on the right in Figure 6, plate 82, shows with the same clarity this spiral winding by three different fibers. The winding is not always absolutely regular. Sometimes, the spiral filament does not enclose all the elementary fibers; one, sometimes two or three, or more leave him; in the latter case, the primary fiber most often divides, at this level, into two new fibers which share the three spiral filaments; one has one, the other two.

By examining the isolated secondary fibers in the fibers of Figures 1, 3, and 5 with a magnifying glass, it will be clearly seen, at certain points along their course, that they are constituted by a straight central fibril around which a second fibril is spirally wound.

These photographs demonstrate, beyond any doubt, that the muscle fiber is not constituted, as has been assumed up to now, by a series of alternately thick and thin discs of two different substances. The clarity of the elements photographed in Plate 82 fully confirms the conclusions previously established.

Does the spiral winding of the entire bundle of secondary fibers, which I had not observed in the previous study, persist in the muscles of the adult? One might believe that it exists by examining certain photographs of fibers, such as those in Figure 3, Plate 81, but this is only an impression that is impossible to relate to a certain objective vision due to the images of multiple shapes that can be produced by the interference of light rays crossing the bundle.

2. Elements contained in the muscles of the fetus independently of the muscle fibers: fruiting bodies, sclerotia, mycelial fibers.

In the muscles of the fetus, either between the fibers or within the fibers themselves, between the secondary fibers, we find a large quantity of ovoid or spherical bodies which are either fructifications or sclerotia; the nature of certain elements can be easily determined. But for others, it is difficult to specify whether they are fructifications or sclerotia.

The bundles of secondary fibers give rise to fine spherical spores of 2 μ to 2 1/2 μ, very refractile, non-basophilic, which can be distinguished in their thickness or on their edges (Fig. 3, pl. 83); they sometimes reach a greater diameter (5 μ, fig. 6, pl. 83).

Other oval, pear-shaped, etc. formations have the same shape and structure as the large spores carried by the mycelial elements of the cerebellum. Some of these elements are placed directly at the end of a secondary muscle fiber, often immediately after it leaves the bundle; it is indeed a secondary fiber that carries such an element, because the presence of the spiral fiber is detected there (Fig. 3, 5, pl. 83).

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Other times, the secondary fiber has a longer path outside the bundle (Fig. 5, and 8 pl. 83) and may present a dilation preceding the ovoid element (A, fig. 8).

Are these formations sclerotia emitting the secondary muscle fiber that ends there or spores? I think that they are fructifications (spores or gonidia) intended to form sclerotia by their agglomeration; this appreciation is justified by the following fact: in the muscle we find large incompletely differentiated mycelial branches whose branches appear to form bundles of secondary fibers; such a branch is represented in figure 11, plate 83; it emits fine branches (at the bottom and left) which are secondary muscle fibers characterized by their spiral winding, and two large branches of which, one (the one on the left, half the height of the figure) broken, shows its fibrillar structure. The fibers are therefore born (at the bottom of the figure) in the large mycelial trunk; Now, one of them (at the top of the figure) has a ball which, therefore, is a spore, and preceding it, a lanceolate dilation identical to dilation A in figure 8. The oval element which ends the latter is therefore indeed a spore.

Moreover, sclerotia have a very different shape from spores as we will see.

Figures 10 and 11 show the large quantity of large spores which form between the bundles of secondary fibers.

3. Origin and mode of formation of primary muscle fibers and secondary fibers.

One of the modes of formation of secondary fiber bundles, which appears to be the normal mode, has just been explained. Indeed, in the mass of strongly colored bundles, we find very pale ones, still hyaline, in which we can barely distinguish the secondary fibers (Fig. 1 and 4, pl. 84). They have the appearance of both connective bundles and *Mucor* filaments, the wall of which would constitute the sheath of the young bundle; in fact, these bundles divide (Fig. 1) and emit branches (Fig. 4).

Between the bundles there are sclerotia of various shapes; Figure 1, Plate 84, shows a large round mass emitting two fine germinative filaments; Figure 2, Plate 84, a larger sclerotia emitting a large branch which could be the origin of one or more bundles. Figure 3 shows sclerotia of other shapes from which the bundles appear to emanate directly.

In large masses of muscle fibers, sclerotia of various shapes, round, oval, spindle-shaped, etc., are clearly distinguishable, often of very large dimensions; others, often fragmented, are distinguishable on the edges of masses of fibers. They obviously emit muscle fibers or the mycelial trunks that form them; no photographs are given here due to the difficulty of making them appear through the mass of muscle fibers.

What is missing from this study, which I have not pursued further, is the determination of the nature of the first bundles giving rise to the spores whose agglomeration will constitute the sclerotia that form the muscle mass. Do these first bundles emanate from connective tissue or nerve filaments? Are they an extension of the nerve fibers?

Consequences of the determination of the nature of muscle fibers.

The problem that was the main object of the anatomical study of muscle fiber is, thus, solved; the primary muscle fiber is a complete organ formed of a membranous cylinder, the sarcolemma, an expansion of myelinated nerve fibers containing a bundle of elementary fibers each consisting of two fibrils, one straight axial, the other spirally wound around it.

The myelinated fiber is a mycelial filament of mold of the *Mucor* type; the sarcolemma is its termination. The muscle fibers enclosed in the sarcolemma are also mold elements.

These facts constituted material proof that the functional organic element of muscle is made up of mold elements taking a special arrangement adapted to the function.

This conclusion led to another:

The mold elements observed in the connective tissue, muscles, nerves, spinal cord, and brain, and constituting all of these organs, are not parasitic elements, but the normal anatomical elements constituting these tissues or organs.

* * *

Thus, the crucial fact which constituted the final conclusion of this research and whose ignorance was the cause of the enigma which appeared after the previous studies relating to connective tissue, nerve fibres and cells, gradually appeared:

***The entire animal organism is made up of an organized mold*.** It seemed obvious that since the main organs-nerve centers, nerves, muscles, connective tissue-were made up of an organized mold, the other organs-liver, spleen, digestive glands, reproductive organs, etc. - must have the same constitution.

The following research will provide the justification. This fact constituted the explanation for certain phenomena or led to consequences that we will examine successively.

1. *Organic mold specific to animals.* It has previously been demonstrated that, when cultivated on agar, the pulp of all the tissues of an animal gives rise to identical gonidial forms of the same species of mold. The tissues of all individuals of the same species give rise, by in vitro culture on agar at approximately 20°C, to the same species of mold and its same gonidial forms.

This is therefore the organic mold characteristic of the species, that is, specific.

2. *Ancestral form of animal organisms.* *It is therefore also, at the same time, the ancestral form that the animal species had at the beginning of its organization and evolution*, unless, during this evolution, it was adulterated by crossing with other molds (progressive hybridization) or by progressive chemical modification by external elements (e.g., food), its specific characteristics being certainly linked to its chemical constitution.

The fact that every living being, animal or plant, is constituted by an organized mold, and the fact of being able to easily isolate from this being the elementary or ancestral mold that constitutes it, definitively and materially establish the progressive evolution of living beings and the link between their primitive form and their current form.

3. *At the death of an animal organism, its living matter does not die; only the organization of its matter gradually disappears*. - A tissue isolated from the body that formed it, does not die, but gradually loses its organization and form, with a speed that depends on the new conditions in which it is placed.

If it is in these conditions as close as possible to those in which it lived in the organism, it can, more or less, maintain its form for a certain time; this is the fact observed in tissue culture, an improper term, because a tissue can only function and vegetate in its normal environment, the circulating blood; outside this environment, its form always ends up being altered and it suffers the fate indicated later.

If it is placed in temperature and environmental conditions very different from the normal conditions of the organism, the tissue rapidly changes form and its organic mold returns to the ancestral type, that is, to its hyphomycete type.

When the organism itself dies, its tissues suffer the same fate; the vegetation of the organic mold changes form and returns to its ancestral type, which is still a living form of organic matter, a form capable of indefinite reproduction.

It therefore does not return to the state of inert organic or inorganic matter as was believed. This explains why normal phenomena can continue for a certain time in the tissues. The muscles continue to contract and the liver to produce glycogen. When liver pulp is cultivated in broth and its pure culture is replanted, it still has the property of transforming glycogen into sugar.

4. *The putrefaction of the animal body after death is not due to the proliferation and dispersion of intestinal microbes.* **-**It is due to the organic mold itself, whose elements, zymospores, bacilli, etc., exist at all points of the organism and cause putrefaction.

5. The animal organism being made up of an organized mold, this forms agglomeration masses or germinative masses like any other mold; these are the normal leukocytes of the organism.

The identity of form that I had observed between the normal leukocytes of the organism and the heterogeneous leukocytes formed by infecting bacterial agents (that is, by heterogeneous molds) is therefore fully justified.

6. Gangrene of the limb, following a ligature (tourniquet) or the section of the main artery, is due to the change in form of the specific organic mold which, its normal nutrition being suppressed, resumes the ancestral form and spontaneously evolves into a bacterial culture.

The heterogeneous molds (permanent mycoses) existing in the limb also develop, in this case, a bacterial culture. The septic vibrio thus develops spontaneously in the ligated limb and not through external contamination.

7. The fatty transformation of cadaveric tissues is due to the persistence of a normal property of organic mold, the transformation of albuminoids into fat.

8. Since molds are the organisms that give rise to bacteria, and the animal organism is made up of an organized mold, *a bacterial culture can therefore arise spontaneously in the latter.*

Certain animal diseases, which have been considered infectious because bacterial elements have been found proliferating in the organism, are therefore autogenous diseases, due to a transformation of the suffering organic mold (diseases attributed to B. coli, pneumonia, bronchitis, liver and kidney diseases, etc.).

9. The in vitro culture of cancerous tissue from the human species gives rise to the specific organic mold of humans. Cancer is therefore an autogenous disease of humans due to a change in the vegetative form of their organic mold.

10. The transformation of the human tuberculosis bacillus into a hyphomycete gives rise to the gonid forms of the organic mold of man. Tuberculosis is therefore, in humans, and at least in certain cases, a spontaneous disease due to a vegetative transformation of the suffering organic mold.

11. The plasma or serum of animals, the substance of all their tissues, or *the materials that are extracted from them*, without heating them to 100°, contain, alive, the reproductive elements of their organic mold.

Injected into the body of another animal, these products bring germs of a mold which vegetate there first in the bacterial state, then subsequently in the mycelial state, always producing the anaphylactic state with a mycosis of indeterminate duration; This infection is called serum disease when it comes to serum.

When we inject a patient with a curative or preventive serum, we therefore administer, in addition to the so-called immunizing elements, the elements of the organic mold of the animal which provided the serum, generally that of the horse.

* * *

I confine myself to these general indications, because there are hardly any questions interesting the biological sciences, in general, whose determinism is not profoundly affected by knowledge of the nature of the animal organism.

Currently, we must examine other consequences that seem to be necessary.

If animal organisms are constituted by an organized mold whose ancestral form is a hyphomycete, that is, a fungus classified in the plant kingdom, it follows that plant organisms, a tree, a herbaceous plant, a moss, etc., must also be constituted by an organized mold.

The verification of this induction quickly demonstrated its accuracy and led me to a summary study of the anatomical constitution of plant organisms, which will be found exposed in its place. This verification will demonstrate that direct examination of tissues allows one to directly detect the vegetation of the specific organic mold and that the culture on agar of any portion of plant tissue reveals its organic mold in the form of the ancestral hyphomycete.

By this process, the gonidial forms of the organic mold specific to a large number of plant species were isolated. At the same time as this isolation, I investigated and obtained, for a certain number of species, the transformation into hyphomycetes of the parasitic agents of their diseases.

The consequences of these determinations were of capital importance for the etiology and knowledge of the nature of animal and plant diseases, because:

1. In the hyphomycete forms of the parasites causing the diseases of any plant species, I recognized the gonidial forms of the organic mold of this species, which meant that these diseases are autogenous and not parasitic.

2. In the hyphomycete forms of the organic mold of plant species, I successively recognized the gonidian forms of pathogenic molds causing diseases with heterogeneous germs: typhoid fever, diphtheria, tetanus, measles, scarlet fever, smallpox, etc. Plants are therefore the original source of a large number of viruses of human and animal diseases; the other viruses have as their original source the organic mold of animals whose species were determined.

Thus, the knowledge was acquired that could effectively guide the prophylaxis of human and animal diseases.

I will stop here with these considerations to return to the methodical presentation of the questions treated in this work.

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I will first complete, by the study of the liver, the spleen, the reproductive organs and the elements of the blood, the knowledge relating to the specific organic mold of animals, knowledge that will be indispensable for the study of other questions such as: the normal physiological phenomena of the organism, blood coagulation, the state of anaphylaxis, the mechanism of immunity, etc.

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VI. Study of the constitution of the cardiac muscle.

This study was made on the rat heart using preparations obtained by dissociation, dry, and stained with dahlia or impregnated with silver.

From the examination of these preparations, it appears that the heart is made up of enormous bundles of mold fibers (P. 85, A, fig. 1; A, B, fig. 2; A, B, fig. 3) dividing into smaller bundles which anastomose with each other (B, C, fig. 1; C, D, fig. 3).

If we examine the elementary constitution of the large bundles, we see that they are formed by other smaller ones, each consisting of a few secondary fibers held together by a spiral fiber identical to the secondary fibers. Figure 1, Plate 86, shows a series of bundles A, B, C, D, E, F, each having its own spiral fiber and anastomosing with each other. The large bundle H, provided with a single spiral winding, divides into two bundles A and B, each having its own winding. The spiral winding is also visible in Figure 5, Plate 84.

Figure 2, Plate 86, shows the same arrangement.

The elementary fibers are made up of a spiral filament wound around a straight axial fiber; these two filaments can be distinguished in fiber A, Figure 4, Plate 87, examined under a magnifying glass.

Furthermore, we will notice, between the bundles of elementary fibers, fruiting bodies identical to those already studied in other muscles (Fig. 1, pl. 86).

There is therefore no essential difference between the heart muscle and a striated muscle of locomotion. The main difference lies in the arrangement and anastomosis of the secondary bundles.

We find in the cardiac muscle the other particularities that I have already noted in the other muscles.

The objective striation of the sarcolemma is made up of spiral fibers emitting a multitude of fine ramifications bearing the spiral and sporiferous appendages I have described (Pl. 87, fig. 1 (gr. 610), fig. 2 (gr. 1100)). The sarcolemma therefore has the same constitution as the fibers of the muscles of locomotion.

The constituent fibrils of the elementary muscle fibers themselves bear the same appendages (Figs. 3, 4, 6 (gr. 610), pl. 87).

Between the muscle bundles (A, fig. 2 and 4, pl. 86) and in their vicinity (A, fig. 4, pl. 85) is found a coagulated substance which often makes observations difficult; it is

made up of liquid flowing from the muscle and which has carried away an infinity of spores and zymospores.

In summary, the heart is made up of long, branched bundles of muscle fibers; these fibers are made up, like the fiber of the muscles of locomotion, of an identical membrane (sarcolemma), containing a bundle of secondary fibers each made up of two fibrils, one straight axial, the other spiral, winding around the previous one.

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VII. Study of the constitution of the liver.

The purpose of this study is not to provide a detailed and complete description of the elements constituting the liver; the purpose is only to show:

1. That the constitution of the liver is different from that taught to us by histology treatises.

2. That the liver is a gland constituted, in principle, like other glands, such as the pancreas, for example.

3. That the liver is not constituted by isolated elements, the cell and the hepatic lobule, but by elements which remain in *constant and distant* relation with the mycelial elements and the distant germinative masses which gave rise to them.

4. That the elements of the liver are elements of the specific mold constituting the organism.

Mycelial framework of the organ.

The constitution of the liver cannot be fruitfully studied on sections of the organ; on these sections, one would have as little chance of perceiving the constitution of the organ and the arrangement of its elements as one would have of guessing the shape of a chair by examining a horizontal section of its legs made ten centimeters from the ground.

This study must be done on pieces of the organ spread by crushing on a single plane *without fragmenting them* as much as possible.

All the photographs reproduced here were taken from preparations obtained by crushing a piece of guinea pig liver tissue, rapid drying by heat, and staining with dahlia.

On such preparations, we find large islets having the appearance shown in Figure 1, Plate 88. On only one of these islets, we can see the constitution of the hepatic gland. Figure 1, Plate 88, represents a large trunk or mycelial bundle A, B, emitting multiple ramifications.

Following it from the letter A, we see it emitting ramifications ending in a bushy arborization whose branches are terminated by rounded basophilic bodies. This branch forms a powerful, dense, very ramified arborization, in which we can distinguish under a magnifying glass rounded masses formed by the union of several basophilic elements: these are the undissociated hepatic lobules.

By examining under a magnifying glass a part of such an arborization photographed at a higher magnification, one can distinguish at the end of the branches formations identical to the encapsulated balls of the cerebral hemispheres and the cerebellum, possessing a large, strongly basophilic central nucleus: these are hepatic cells.

Plates 88 and 89 contain photographs of different aspects presented by the islands of hepatic substance in the crushed preparations and at different magnifications.

Hepatic lobules.

If we now examine the terminal arborizations of the mycelial branches at a higher magnification, we see that each branch or each terminal ramification is constituted (Fig. 1, pl. 90) by a cluster of encapsulated balls which are hepatic cells (many of them deformed by crushing); we note, moreover, that some are grouped in clusters of 4 or 5 undissociated elements (A, fig. 1); these are hepatic lobules which can be better seen in figures 2, 3, 4 of plate 90. One of them, B, is very clear in figure 4.

The hepatic cells are not independent and isolated; they are re-linked by a mycelial pedicle to the mycelial framework of the organ.

By searching for isolated elements around the edges of the islets, that is, in the thinner regions where many elements have not been compressed and deformed, we find encapsulated balls or intact liver cells. Their different aspects are shown in Figs. 2, 3, 4, and 5 of Plate 91.

They are presented in two types: some terminal and pear-shaped (A, B, Fig. 5), others in the form of a bead on the mycelial filaments and in the form of a short, wide spindle, pointed at both ends (Fig. 2).

The fine mycelial network surrounding the nucleus is very fragile; therefore, many elements are reduced to the latter by crushing.

We also note other formations, notably those in the form of an elongated and flat spindle (Fig. 1, pl. 91), which have already been described on the occasion of the initial search for mold elements in the liver and other organs.

It is clearly seen in these different figures that, even in one group of elements (A, fig. 4, pl. 91), the liver cell is located at the end of the long mycelial filament that formed it.

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Constitution of liver cells.

Examined at high magnification, the liver cells show a constitution identical to that of the encapsulated balls of the cerebellum. They are formed by the division of the formative filament into a large number of branches, themselves branched and bearing a large number of spiral spore-forming appendages. These branches form an areolar tissue in the middle of which arises a large, spherical, very basophilic nucleus (D, fig. 2, pl. 92). This nucleus results from the mass, in the center of the cell, of the basophilic protoplasm of the filaments of the areolar tissue. We see, in fact, that a large number of filaments penetrate the ball and contribute to the formation by flowing into it, either directly or rather in the form of spores, their basophilic protoplasm.

In Figure 4, Plate 92, we see a central ball whose formative filaments are very visible (B, B). In these nuclei, detached from cells torn by crushing, we often still see the sinuous path of the formative filaments that have remained visible there (Fig. 6, 8, Pl. 92), or we still see the trace of their insertion at the periphery (A, Fig. 6). In the preparations we also note:

1. Balls to which mycelial filaments adhere and which take on the forms photographed in Figures 9, 10, 11, Plate 92. It has not been possible to discern whether these are special elements in formation; it seems more likely that they are deformed liver cells brought into this state by the crushing of the tissue and the sliding of the glass slides over each other, or even liver cells in the process of disappearing.

2. Spores of form A (Fig. 7, pl. 92). These spores are formed by branching of the mycelial stalk A; the branches of this stalk form fine interior spores already visible (zymospores).

These observations prove that the liver, like the other organs, is made up of the vegetation of a mold: the specific mold of the animal.

The hepatic cell is a formation of the same type as the encapsulated balls of the cerebellum; because of the prominent role these elements play in the liver, of which they constitute the greater part of the mass, it must be concluded that, in the cerebellum and the cerebral hemispheres, these elements play an equally crucial role.

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The origin and role of cholesterol are unknown. Some physiologists believe it is of plant origin and introduced into the animal body with plant elements.

Cholesterin exists equally in animal and plant organisms because both are made up of an organic mold rich in cholesterol. It is this body that appears to give basophilic protoplasm its power to absorb basic colors; basophilic zymospores are heavily laden with it. The liver's richness in cholesterol therefore corresponds to its richness in basophilic protoplasm.

If cholesterol stones form in the gallbladder, it is because the liver zymospores contained in the bile clump together there and form large masses; this fact alone would be sufficient to prove the great richness of zymospores in cholesterol.

The liver does not eliminate its cholesterol: the liquid bile secreted by the liver owes its activity, like other digestive juices, to its zymospores, as well as to the other bodies it contains.

I was able to verify the power of cholesterol. If water is added to an alcoholic solution of cholesterol, so as to obtain a very cloudy emulsion of very fine grains of this body, it is seen, at high magnification, that these grains are animated by the Brownian movement and that they strongly fix the basic colors. If a little of this liquid is added to a very fine emulsion (obtained mechanically) of olive oil in distilled water, it can be seen, by patiently observing and following the fine particles of cholesterol in their movement, that one of them arriving against a globule of oil, much larger than itself, splits it instantly into two globules which separate.

The Brownian motion of the cholesterol globule probably imparts a gyration movement to the oil globule and at the same time marks, on a diameter, a cutting line along which the globule splits into two parts due to the rotational movement imparted to it. It is likely that the explanation of the emulsifying power of bile is due to the cholesterol it contains in the form of zymospores and that it is the phenomenon just described which achieves the emulsion, probably facilitated by the other bodies it contains.

The liver tissue, reduced to a fine pulp and cultivated on agar, gives rise to various gonidian forms of the organic mold that constitutes it.

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Before concluding this study of the liver, it is appropriate to examine a particularly important point in the constitution of this organ.

The large mycelial bundles from which emanate the filaments that carry the encapsulated balls, that is, the hepatic cells, are part of the fibrous framework of the organ.

They must therefore come from the suspensory ligament of the liver; this, which is considered to be of a connective nature, would therefore be constituted, in reality, by the mass of mycelial bundles that will constitute the organ; in fact, these bundles radiate

into the liver, of which they constitute the connective framework and form, at their terminal ends, the hepatic cells.

It is therefore necessary to investigate where the bundles of fibers that constitute the suspensory ligament originate.

Are they a direct or indirect emanation of nerve fibers from the centers or the sympathetic, or do they come from distant sclerotia?

The same problem arises for other organs, spleen, kidney, testicle, ovary, etc.,whose fibrous framework is in reality the mycelial mass which constitutes, at the ends of the branches, the so-called noble elements of the organ.

VIII. Study of the Constitution of the Spleen.

The subject of study was the sheep spleen. Fragments of spleen are crushed between two glass slides; they are dried while hot, stained with dahlia, and then washed to remove excess dye.

In such preparations, the spleen tissue is distributed into islands in which are found large mycelial trunks or bundles which, with their ramifications, constitute the fibrous framework of the organ (Figs. 1, 2, 3, pl. 93). The mycelial branches emit laterally a large quantity of fine mycelial filaments (Fig. 3, pl. 04) which bear, laterally and at their ends, various formations.

1. These formations are made up, for the most part, of rounded or pear-shaped bodies (Fig. 2, 3, 4, pl. 110), generally lacking a nucleus or having a nucleus very small in relation to the mass. These bodies are made up of multiple ramifications of the peduncle; these, sinuous, intertwined, bear fine spores.

2. Some formations having a constitution identical to the preceding ones take on varied forms, that of a spindle, an eggplant, etc., etc. (Fig. 4, pl. 110).

3. Other similar formations possess a large, round central nucleus and have a constitution identical to the encapsulated balls of the foic.

4. At the end of certain filaments, we find rounded formations possessing an enormous nucleus, sometimes two and even three, occupying almost the entire mass; the areolar constitution of the mass surrounding the nucleus is no longer distinguishable,
although, quite obviously, it existed before the formation of the nucleus. These formations appear identical to mono- or polymorphonuclear leukocytes (Fig. 4, 5, 6, pl. 110) and seem to be one of their origins.

As for the first two categories of formations, *they are identical to blood globules.* There is no doubt that these perithecia, detached from the tissue of the spleen and carried into the circulation, constitute blood globules because in the preparations of the latter, made by rapid dissociation and sufficiently colored by dahlia, we still see, adhering to the globules (at least in the frog), the fine peduncles which formed them and which, in the spleen, connect them to the mycelial framework of the organ (Fig. 1, 2, pl. 108; fig. 1 and 4, pl. 109).

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Moreover, to complete the proof of this origin, we find, alongside the blood globules, the spindle and aubergine formations (A, B, fig. 1, pl. 108) of the second category also still have their peduncle.

Moreover, when we examine these formations, either in the islets of crushed tissue or among the detached elements around them, we find elements of all sizes, notably elements of the size of the hematoblasts and having the same constitution as them. The *hematoblasts, like the blood corpuscles*, therefore come, at least in part, from the spleen (Fig. 2, pl. 109).

5. Certain fine filaments, emitted laterally by the mycelial branches, are terminated by formations taking the most varied forms, such as spindles, leaves of various plants, etc.; sometimes, they end in a point extended by a filament carrying a large spore; some of them have the form of trypanosomes (Fig. 1, 2, 3, pl. 94). Like blood cells, these formations can fall or are destined to fall into the circulation; in the rat, they constitute the elements considered to be trypanosomes.

I will not dwell here on the description of these elements, which has already been made previously in the spleen of the rat, the mouse, and various other animals.

The elements just described appear distinct in the lower part of Figure 4, Plate 94, due to the method of preparation by crushing the pulp of the spleen. In reality, the rounded and pear-shaped formations are grouped into islands or lobules of five to six elements visible in the upper part of the same figure.

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IX. Study of the Constitution of the Testicle.

The following observations relate to the guinea pig testicle and were made on preparations obtained by crushing a portion of the organ between two glass slides, rapid drying, and staining with dahlia.

Mycelial Framework of the Organ.

This organ is made up, like the liver and the spleen, of a fibrous framework that is mycelial in nature. Large mycelial trunks or bundles branch out to form arborizations of filaments that take the form of enormous spindles (Fig. 1, plate 95 and Fig. 1, plate 96). Figures 2 and 3 of Plate 95 show various aspects of the preparations and the ramification of the mycelial trunks Each mycelial arborization emits, on all its branches, lateral ramifications carrying, in clusters, a series of formations constituted by a branch rolled up on itself in the shape of a crook which are spermatoblasts. Two of the constituent branches, showing the numerous lateral branches which they emit, are represented, figures 1, 2, 3, plate 97.

For one of them the photograph has been developed to two different degrees to show that it is indeed a mycelial branch whose traces of partitioning are visible in A, B, C, D, E (Fig. 1).

In Figure 2, Plate 96, there are also three other smaller branches, whose structure can be easily studied under a magnifying glass; this examination will show that they have an appearance identical to those of the dendrites of the pyramidal cells of the hemispheres, and that the varicosities they present are due to flat, short-stalked spores, born on them and pressed against the filament.

Clusters of crooked formations or spermatoblasts.

Figures 4 and 5, plate 97, show interesting features, notably the mode of formation of spermatoblasts by the curling of a mycelial filament into a crook. The clusters of perithecia cannot be studied in large mycelial arborizations where they are too compact; they must be sought in the regions of the preparation where they have been isolated by crushing and where their shape is not altered.

Figures 1 and 2, plate 98, represent two such regions containing mainly clusters of forming and incompletely developed spermatoblasts (B, fig. 2), and clusters in a state of complete development (E, fig. 2, pl. 98). Figures 3 and 4, plate 98, and 3 and 4, plate 99, show isolated clusters in a state of incomplete development; Figure 2, plate 99, contains a mature cluster.

One of the figures in plate 99 (Fig. 1) is of particular interest. We see a mycelial branch that divides into two others, B and C, segmented into distinct and ramified articles like the mycelial filaments which, starting from a vein of the leaf of *Althea officinalis* (Fig. 1, 2, pl. 137), for example, divide to spread out in the leaf parenchyma that they will constitute. Branch B, for example, ramifies into three articles; the left primary article E

continues with another article F which emits two others, one of which G will bear a cluster (of which only the beginning of the pedicle remains) and the other of which is extended by a fourth K. The central primary article constitutes the cluster of perithecia I; the right primary article bears two articles but is broken at this level.

Thus, organic mold presents, in animal organisms, devices identical to those it presents in plants, in the parenchyma of a leaf, for example; multiple examples of the same kind could be noted, I have indicated another one concerning the dog's spleen; others will be found in the presentation of the constitution of plant tissues.

Constitution of perithecia and spores.

It remains for us to examine the constitution of spermatoblasts and the formation of spermatozoa.

The constitution of spermatoblasts can be studied under a magnifying glass in most of the photographs in plates 96, 97, 98, 99, 100; it is very apparent in figure 2, plate 99. They are formed by the crooked winding of the ramuscle of the cluster which forms them; this filament winds itself over a complete turn or one and a half turns and gives rise, on this circular path, and on its internal face, to 3, 4 or 5 large, slightly oval, short-pedunculated spores; each spore is itself formed by a ball of the ramifications of its pedicle (Fig. 2, plate 99).

Formation of spermatozoa.

It is in these spores that spermatozoa develop.

Their formation can be easily studied in regions where numerous isolated spores exist; we observe:

1. That the spore expels a substance forming a halo around it, A (Fig. 3 and 4, pl. 100).

2. That part of the spore's contents contracts and that, as a result, we observe two very distinct regions: the contracted region C (Fig. 3 and 4) which will lead to the formation of the spermatozoon's head and the abandoned region B.

In Figure 4, we see the head C of the spermatozoon fully formed and already halfway out of the spore. Examining the abandoned part B of the spore, we see that it is made up of the hyaline substance of the coiled filaments that constituted it and whose baso-philic protoplasm has migrated into the head of the spermatozoon. Indeed, the residual spore no longer takes on the bright coloration of spores that have not yet evolved.

If we examine with a magnifying glass a spore still included in a spermatoblast (A, fig. 1, pl. 100) and forming a spermatozoon, we see that the region which already takes the form of the head of the spermatozoon contains a group of small basophilic balls (1 1/2 to 2 μm), very stainable, which are fine spores formed on the coiled filaments constituting the large mother spore; these fine spores, being detached from the filaments, each emit a *flagellum*; two or three of them can be distinguished in spore A; proof of this fact is, moreover, easy to give, because there always exists, in a studied field, a fairly large number of these fine spores of 2 μm isolated and coming from the fragmentation of the large spores by crushing maneuvers; These fine spores seen in isolation are all provided with a fine and long *flagellum* (Fig. 1, 3, 4, 5, pl. 100; fig. 2, pl. 99).

The contraction of the basophilic protoplasm is therefore done by fusion of the elementary spores formed in the mother spore; it results that the head of the spermatozoon is constituted by the fusion of a certain number of fine flagellated spores of 1 to 2 μm (I counted from 12 to 20), and it seems that there may exist a greater number, each emitting a *flagellum*.

The single *flagellum* of the spermatozoon is formed by the fusion of those of the elementary spores constituting the head.

Summarizing these observations I will conclude:

1. That the testicle of animals is formed by the mycelial vegetation of the organic mold constituting the animal;

2. That this mycelial vegetation forms clusters of spermatoblasts in the testicle.

3. That spermatoblasts are formed, like certain perithecia of molds, by the winding of the end of a mycelial filament on itself.

4. That each spore of the spermatoblasts is itself constituted, like the gonidia of molds (those of the *Peronospora*, for example), by the ramification of the gonidiophore and the coiling of its branches.

5. That the spore forms, within itself, fine flagellated spores identical to the flagellated zoospores formed by a gonidia of *Peronospora*: the former are therefore comparable to zoospores, just as the large spore that forms them is comparable to a gonidia of a *Peronospora.*

6. That the spermatozoon of animals is constituted by the fusion of a group of zoospores from the gonidia formed by their specific organic mold in the organs specially assigned to reproduction.

Study of the constitution of the ovary.

This study cannot, unlike the previous ones, be carried out fruitfully by the sole examination of preparations obtained by crushing the tissue. It requires the examination of thin sections of the organ, which alone can provide information on the mode of egg formation.

For a first series of observations, made on preparations by crushing, I turned to the guinea pig ovary; the observations relating to the mode of egg formation were made on sections of the dog's ovary.

Guinea pig ovary.

I will be brief on its constitution. The preparations by crushing show three types of elements:

1. Large spores carried by long mycelial filaments (Fig. 1, 2, 3, 4, 5, pl. 101). These spores are all formed by the entanglement in a ball of the ramifications of their pedicle; they are sometimes arranged in a cluster at the end of the filaments.

2. Large mycelial masses formed by the filaments rolled into a ball and carrying spores similar to the previous ones. These are the perithecia in which the eggs are formed. These opaque masses are difficult to observe (Fig. 6, pl. 101).

3. Elements identical to the encapsulated balls, carried by short mycelial pedicles having one or two times the length of the element, often less, and born on lateral branches of mycelial twigs (A, B, fig. 7 and 8, pl. 101). These balls, pear-shaped, are constituted by the sinuous ramifications of the pedicle of the element entangled in an areolar tissue. In the center of this tissue is formed a ball of an identical tissue, but whose mycelial filaments are more clearly wound in a ball to form a regular spherical mass which is strongly basophilic; the filaments of this mass themselves carry small very basophilic spores; some of these fuse together to form one or more small very regular spheres fixing the basic colors more energetically than the rest of the ball; in certain nuclei, the masses thus formed and melted together take on such a volume that it seems certain that, at a given moment, the whole of the central ball is occupied by a uniform mass of basophilic protoplasm.

The observations made provide no information on the fate and role of these elements.

Ovary of the female dog.

All the photographs recording the observations made (Pl. 102, 103, 104, 105, 106) were taken from the same section of a female dog's ovary.

The first examination of this section, made at low magnification, shows the known constitution of the ovary.

1. A peripheral part (A, fig. 1, fig. 2, pl. 102) formed of wavy fibers, often roughly parallel, winding in a whirlwind around the points where the eggs form, and small masses or large basophilic granules.

2. A central part (Fig. 3, pl. 102) formed of large pear-shaped or spherical cells, packed close together and equipped with a large nucleus containing one or more nucleoli.

Here we find the two elements that we observed in the guinea pig ovary; the wavy fibers of the external part are mycelial filaments bearing basophilic spores, which are the multiple granulations scattered between the fibers; the cells of the central part are the encapsulated balls described previously. By examining the enlarged central part, we see the mycelium, from which the ramifications that form them, originate, traveling between these cells, or encapsulated balls (Fig. 4, pl. 102). Certain encapsulated balls are visible enough in Figures 3 and 4 to show the identity of their constitution with the encapsulated balls of the guinea pig, and it is unnecessary to describe them again.

In the external region of the ovary, we see the wavy fibers winding in a whirlwind around the points where the eggs are formed.

In reality, these fibers, which are mycelial filaments of a mold, thus coil to form a perithecia identical to those formed by molds vegetating in vitro or in the external environment; a perithecia of the animal's specific organic mold forms one or more eggs like the perithecia of a mold cultivated in vitro.

Formation of perithecia.

The wavy fibers of the external region of the ovary coil in a spiral to form a shell which will be the envelope of the perithecia.

Figure 1, plate 103, shows the beginning of the formation of a perithecia by spiral coiling of filaments appearing to emanate from the mycelial branch A, whose articles are very distinct. Figures 2, 3, 4, 5, contain other perithecia at various stages of their development.

The perithelium is formed, at the very beginning, only by coiled mycelial filaments constituting a mass of variable size; the external part of the perithelium appears to increase by the coiling of new filaments, while, in the central part, spores are born on the filaments (Fig. 4). From this moment the formation of the egg begins to become apparent.

Formation of the egg.

Various stages of this formation are fixed in the photographs of plates 104 and 105; they are the following, from the beginning of the formation of the perithelium:

1. ***Beginning of the formation of the perithelium*** (Fig. 1, pl. 104).

2. ***Growth of the perithelium*** by the coiling of filaments on the outside and the formation of spores on the inside (Fig. 4, pl. 103).

3. ***Formation of spores*** by the internal filaments of the perithecia; as this formation of spores takes place, the mycelial filaments that form them take on a radiant arrangement (Fig. 2, pl. 104). These spores form in strings at the ends of the filaments; these take on, in some of them, a bulging appearance like that of a sterigmate (Fig. 2, pl. 104).

The emitted spores detach and become free in the center of the perithecia.

4. ***Germination of the central spores.*** They each emit one or more sinuous mycelial filaments, bearing appendages ending in a small basophilic spore and forming by their entanglement an areolar tissue. This tissue forms, at the center of the perithecia, a ball of increasing volume (Fig. 4, 5, 6, pl. 104, and 1 to 6, pl. 105). The beginning of this germination of the spores is visible at the center of the perithecia, in figure 2, pl. 102. As this mass of filaments develops, the spores which form it are pushed to the periphery of the perithecia. This mass of areolar tissue will constitute the nutritive yolk. The fine spores formed by the sinuous mycelial filaments constituting the areolar tissue are the bodies which, in the egg, and in all tissues in general are called mytochondria. They are zymospores of organic mold.

5. ***Formation of the vitelline membrane*****.** When the central mass of areolar tissue has acquired a certain volume, the peripheral filaments, which appear richer in basophilic protoplasm (zona pellucida, fig. 2, pl. 105), are packed against each other (Fig. 3, pl. 105) to form a porous membrane (Fig. 5, pl. 105, then fig. 4) through the holes of which pass or engage the fine filaments emitted by the spores remaining outside the membrane, and whose vegetation will continue to increase the volume of the yolk (Fig. 4 and 5, pl. 105).

6. *Complete growth of the egg: radiant corona*. The external spores, thus connected and adherent to the egg by their germinal filament which crosses the vitelline membrane, constitute the corona radiata (Fig. 3, 4, 5, 6, plate 105) which will disappear when, the protoplasm of the spores having passed into the interior of the egg, they will disappear by this fact. Figure 7, plate 105, represents the egg having reached its final size.

Formation of the germinal vesicle.

The preceding observations have not mentioned the formation of the germinal vesicle because, due to the chance of the section, it is rarely involved and visible.

Two photographs (Figs. 4 and 6, pl. 105) show either the beginning of its formation or a section passing between the center and the surface of the vesicle; this beginning coincides with the formation, at certain points, of small germinative masses, the result of the fusion of the fine spores formed by the areolar tissue. These germinative masses (visible under a magnifying glass in figures 4 and 6, pl. 105) emit mycelial filaments which are arranged in such a way as to circumscribe, by a new membrane formed of areolar tissue, a small sphere in the center of the large one limited by the vitelline membrane.

It is probable that the filaments of the neighboring areolar tissue contribute to this formation. The limiting membrane of the germinal vesicle is visible, with the spore-bearing appendages it carries, in the figures on plate 106. In the latter, three ovules are photographed: one in figures 1 and 2 (at two different focuses), another in figures 3, 4, 5 (at three different focuses), the third in figure 6.

Study of these photographs with a magnifying glass shows:

1. That filaments of the areolar tissue outside the germinal vesicle pass through its membrane and form spores inside it. On the other hand, we see filaments starting from the spores of the radiant corona, that is, external to the vitelline membrane, crossing the vitelline, penetrating through the membrane of the ovule and branching out into it. The spores of the radiant corona therefore continue to flow their contents into the ovule.

2. That the interior of the germinal vesicle is made up of mycelial filaments forming large areoles and constituting a kind of supporting framework.

3. That the mycelial filaments of the germinal vesicle emit highly basophilic spores which fuse and form the germinal spot. This must be made up of a large number of

these fine spores, because 16 or 17 can be counted under a magnifying glass in the germinal spot of Figure 4, Plate 106, although the egg that forms it is not yet mature.

Summarizing these observations, we can conclude:

1. That the egg of animals is born like that of molds, in a perithecia; that this is formed by the mycelial filaments of their specific organic moiety.

2. That the yolk is formed by a mass of mycelial filaments.

3. That in birds' eggs, the nutritive yolk (egg white) is formed by the liquefaction of a mycelial mass. This fact is of some importance, for it proves that the ovalbumin and ovoglobulin of the egg come from the liquefaction of mycelial filaments, which explains how easily these substances can later reconstitute mycelial filaments in the embryo. From this, we can also draw a useful indication that sheds light on the role and fate of fibrinogen, globulin, and albumin in the blood.

4. That the germinal spot is formed by the fusion of fine spores (from 0.4 μm to 1 μm) formed in the germinal vesicle by the mycelial filaments that constitute it.

X. Study of the Formed Elements of Blood.

The best method for studying the formed elements of blood consists of centrifuging oxalated blood at 1 part 100, taking a portion of the white layer covering the surface of the mass of red blood cells, mixing it with a few drops of saline at 8 parts 1000, spreading this liquid on a slide and drying it quickly on the hot plate; staining with dahlia and washing to remove excess dye.

In such preparations, it is found that the blood contains:

1. White blood cells.
2. Red blood cells.
3. Hematoblasts of 5 and globules or hematoblasts varying in size from 5 to 7 μ.
4. Strongly basophilic bacillary elements of varying lengths, segmenting into increasingly smaller elements until they form micrococci with a diameter equal to the width of the bacilli.
5. Non-basophilic hyaline bacillary elements.
6. Spherical granules of various sizes:
A. Originally refractile, hyaline, or basophilic spherical granules of either 2.8 to 3 μm, or 22, or intermediate or slightly smaller dimensions.
B. Refractile granules of 0.5 to 0.8 μm.
C. Fine basophilic granules of 0.3 to 0.5 μm and even smaller ones of 0.1 to 0.2 μm
D. Granulations of 03 to 005 that appear foreign to the blood.

7. Sinuous filaments, quite long, bearing short-pedunculated basophilic spores of 0μ 3 to 0μ 5.
8. Germinal masses of irregular shapes, of variable size, strongly basophilic and which have nothing in common with leukocytes.

Let us examine these various elements in turn:

1. White blood cells.

Previous observations have shown that they can have several origins, either relative to their mode of formation or relative to their provenance.

A. They are most often masses of agglomeration of the elements of the specific organic mold; but they can also be heterogeneous leukocytes formed by agglomeration of the elements of external infective molds. The normal leukocytes of the animal are formed by the agglomeration of the various species of hyaline and basophilic granules, mentioned above, to which are added basophilic bacillary elements which contribute to forming the nucleus with the other basophilic elements.

B. The normal leukocytes of the organism can come from the spleen, perhaps from other organs; leukocytes of this origin are represented in Plate 110, Figs. 3, 4, 5, 6.

2. Red Blood Cells.

The spleen contains a considerable number of elements identical to red blood cells; they certainly originate, at least in part, from this organ. The mycelial filaments of the spleen emit fine branches whose ends ramify to form an areolar tissue whose mass takes either the shape of a more or less elongated pear, or an almost spherical shape. In the center of the areolar tissue, a nucleus of variable volume is formed by fusion of the spores formed by this tissue.

To easily understand the structure and evolution of blood cells, it is appropriate to take, as a subject of study, large red blood cells, those of the turtle or other reptiles, for example; it is also appropriate to study them on young animals.

The following study was carried out on the blood of an *Alligator Mississipiensis*, aged two or three weeks, made available to me by Mr. Roule, professor of herpetology specialist at the Museum. In the blood of this young alligator, I observed the various forms of the blood cell corresponding to the successive stages of its evolution; these stages are represented in plate 107 and can be easily followed from figure 1, representing the element that has just been drawn into the circulation, to figure 7 representing the element that has reached its perfect state.

The element represented in figure 1 is a large encapsulated cell or ball, probably detached from the spleen and still clearly possessing its formative pedicle A. The areolar tissue of the element is very distinct, as are the fine spores that it forms; the large central nucleus D of the element is quite easily distinguished.

Two other similar elements A, B (Fig. 2), but disorganized, show more clearly the constitution of their areolar tissue and their nucleus. In Figure 3 we see another element A, still possessing its pedicle D, and showing that the basophilic protoplasm of the areolar tissue passes completely into the nucleus; in fact, this tissue has weakly fixed the dye.

Figure 5 shows a more advanced stage; the globule has taken on its oval shape and its pedicle has disappeared, or is often very barely visible, but the areolar framework is still very visible; it becomes less and less so (Fig. 4, 6) and is only barely distinguishable in perfect globules (A, B, Fig. 7; B, C, Fig. 3). This framework becomes partly visible again if adult globules are stained with carbolic fuchsia, which causes hemolysis by swelling of the globule, in localized points, in the form of pockets which burst The torn tissue of these pockets shows the dissociated areolar framework (A, B, C, D, fig. 8, pl. 107). If the globule swells only at certain points and not regularly throughout its mass, it is because the areolar framework resists and only yields at the weakest points where the pockets close.

The blood cell is therefore not made up of a substance enclosed in a pocket limited by a membrane. It is a mass of compact areolar tissue, the constituent filaments of which emit fine spores (zymospores or mitochondria).

This constitution is the same for the red blood cells of mammals. Plate 108 contains photographs of guinea pig blood cells stained with dahlia. The observation was made by two different methods: first, on preparations obtained by rapid fixation of the blood cells on a slide by heat, then staining with dahlia (Figs. 1, 2, 3, 5, 6, 7); second, by adding a little blood to 8% saline water and, in addition, a little dahlia solution. After 25 to 30 minutes, the blood cells have fixed almost all of the dye, and their pattern can be seen if the liquid between the slide and the coverslip is observed at high magnification (Fig. 4).

In this last case the globular framework, which has not been altered by any maneuver, has completely retained its appearance and its form; one can clearly distinguish the fine, short-pedunculated spores, which are born on the sinuous filaments constituting this framework.

In figures 1, 2, 3, 5, 6, 7, plate 108, the form of the elements has been altered; but one sees that they have taken on a form identical to that which they have in the spleen, prepared in the same way, by rapid desiccation; they have resumed their obtuse pear shape; a large number still possess a fragment of their pedicle adhering to the pointed part of the pear.

A certain number of elements have the shape of an elongated pear (A, fig. 1) like many elements of the spleen; others have the shape of an eggplant and are still equipped with their very visible pedicle (B, C, fig. 1); this last element, common in the spleen, clearly shows that the blood cells come from this organ.

The fig. on plate 109 show the considerable quantity of these elements in the spleen. To easily recognize them, the outline of some has been marked with a black line in fig. 4 (B); they will be found in B in fig. 2.

Further proof of this origin is provided by the existence in the spleen of numerous elements of the same constitution but smaller, measuring 4 to 7 microns, and possessing all the forms known to hematoblasts (Figs. 2 and 4). These therefore have, at least in part, the same origin as blood corpuscles.

3. Hematoblasts

A group of hematoblasts, ranging in size from 4 to 6 µm, is photographed in Figure 1, pl. 111. By examining the elements located at the periphery of the mass or those in Figs. 2 and 3, one sometimes sees that they emit fine mycelial filaments; some of these are still adherent to the hematoblasts (Figs. 1-2). These well-developed filaments branch out and bear numerous, fine, very short-stalked spores (zymospores). Leukocytes emit filaments of the same kind.

There are a large quantity of these filaments in the blood. They appear to originate, for the most part, from the germination of refractile spores of 1 to 2 µm. When a little diluted plasma is placed and dried on a glass slide, these filaments are seen to be tangled and forming groups, one of which is photographed in Figure 8, Plate 111. By examining the mass under a magnifying glass, the zymospores carried by the filaments can be clearly distinguished; these are of two types: the finest carry basophilic zymospores of 0.4 µm on average; there are larger ones which carry refractile, non-basophilic spores of 0.8 to 1 µm. This is the origin of at least a part of the zymospores contained in the blood (Serozyme, mittelstück).

4. Bacillary elements

Blood always contains basophilic bacillary elements resulting from the segmentation of long mycelial filaments (Figs. 3 and 5, pl. 111) which are quite easily observed in preparations. These filaments are most likely emitted by strongly basophilic germinative masses (several types of which are represented in Figures 11, 12, 13, pl. 111), because the beginnings of these filaments can be seen at their periphery. The bacillary elements in turn gradually fragment (Figs. 2 and 3, pl. 111) until they form spherical elements (micrococci) having their width as diameter (0 µ. 6 to 0 µ. 8).

These micrococci and basophilic bacilli unite with the refractile spores to form the normal agglomerating leukocytes of the blood; the basophilic elements fuse together to form the nucleus. The basophilic elements also unite into irregularly shaped germinative masses (Figs. 11, 12, 18, pl. 111).

Similar bacillary elements are found in all tissues.

5. Granulations

We have just seen the origin of basophilic granules of 0.4 to 0.5 µm (zymospores) and non-basophilic refractile granules of 0.8 to 1 µm.

When looking with a magnifying glass for zymospores in the various figures on Plate 111, some are found to be barely visible and have a size of 0 µl or 0 µ2. Such small granules can be released by the fine mycelial filaments emitted by the hematoblasts (endomycelial formation) or formed externally on the walls. This is common in molds cultivated in vitro.

In addition, spherical granules are found in the blood, colorless and refractile, or basophilic and more or less colored; some are from 1 µ 1/2 to 3 µ (Fig. 6, pl. 111).

They are refractile or basophilic to a variable degree according to the number of basophilic elements (zymospores) that they have already fixed. They probably result from the fusion of spores of 0 µ 8 to 1 µ; the volume of the latter is further increased by the agglomeration of zymospores of 0 µ 4 to 0 µ. 5. In the preparations, we see that some have fixed a zymospore on the side or two zymospores placed at the two ends of a diameter.

Often, the zymospore is visible in the center of the spore, the latter forming a white halo around the first; this white halo, due to the refraction of hyaline spores, cannot be

confused with that which surrounds gyrating zymospores and which results from the agitation of the liquid.

The fixation of zymospores by hyaline spores is clearer when examining, between slide and coverslip, diluted liquid plasma colored by the dahlia, taking care to focus on the upper surface of the glass slide onto which the spores fall.

A certain quantity of spores and zymospores in the blood must certainly come from various tissues; these are elements that are impossible to differentiate morphologically from the elements formed in the blood itself.

Finally, one will notice in the photographs another type of dark black granules, with a very clear outline, of dimensions varying from 0 μm to the limit of visual perception. These are elements foreign to the blood, brought in, at least in part, by the coloring reagent. Despite careful filtration of the coloring solution, it is impossible to avoid them, because they pass through the filters.

6. Mycelial filaments in the blood

The mycelial filaments emitted by hematoblasts, leukocytes, or spores have already been noted above. In addition, much larger mycelial debris, of very variable shape and length, probably originating from the tissues, are found in the blood.

7. Germinative masses

They have been noted above; various forms are represented in Figures 11, 12, 13, Plate 111. They have irregular and varied size and shapes. They appear to be composed almost entirely of basophilic elements and to have the role of emitting mycelial filaments which segment into bacilli and micrococci.

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XI. Electrical Organ of the Torpedo.

It is known that the electrical organ of the torpedo is made up of a mass of polyhedral prisms, generally hexagonal, placed vertically between the back and the belly of the animal; each of these prisms is partitioned transversely by a series of lamellae, each bearing the name of the electric organ blade, or electric blade. The nerve fibers branch out into the electric blade, which, for this reason, has been considered the active element of the organ.

There is, however, another element that certainly plays an important role in the functioning of the organ. Between the electric blades is a space filled with a very aqueous spongy material, which releases its liquid when placed on a sheet of Duvard paper; we

can guess, from this fact, that it is made up of an areolar tissue trapping water in its mesh. The study of this material is quite difficult, because it is almost completely disorganized by dissociation or crushing.

The study of the electric organ must therefore include that of the material between two blades, in the space that we will call alveolus.

Study of the electric blade

Several authors, notably Ranvier, have described the termination of nerve fibers in the wall of the alveolus; we will show that the fibers do not terminate in the wall but constitute it almost entirely, with the exception only of the very thin layer of connective elements which exists on the dorsal wall of the electric lamina and whose elements come from the wall of the prisms.

Plate 112 contains photographs of the ventral surface of an alveolus of the electric organ of the marbled torpedo (Figs. 1, 2, 3, 5, pl. 12). This wall, well spread on a glass slide, is dried and fixed quickly by heat, then stained with dahlia or impregnated with silver nitrate.

In the preparations stained with dahlia, it is easily seen that the large nerve fibers, which ramify on the surface of the membrane, are mycelial filaments of a mold of the *Mucor* or *Aspergillus* type. These filaments bear dilatations generally containing one large oval intramycelial spore, sometimes two or three. The special appearance of the ramifications is known (Fig. 5, pl. 112).

Some branches end in fructifications in the form of a spherical ball, sometimes oval, which are of the *Aspergillus* type, as will be demonstrated later (Figs. 1, 2, 3, 5). These fructifications are clearly indicated in a figure in Ranvier's *Treatise on Histology* (Fig. 277).

The other branches all emit, laterally, very numerous and fine mycelial filaments, themselves branched, and bearing a considerable number of spiral or simply sinuous appendages, often straight, all ending in a fine spore (Fig. 4, pl. 112); they terminate, moreover, by emitting numerous similar filaments and nearly several successive dichotomous ramifications.

These sporiferous appendages, notably the straight appendages, very clearly visible in figure 4 with their terminal spore, are evidently the fructifications characterizing the gonidian form of the mold in its terminal ramifications.

By examining these ramifications and appendages with a magnifying glass, it will be seen that their form differs significantly from that given by Ranvier (*Traité d'Histologie*, fig. 281) and other authors.

All these fine branches bearing spore-bearing appendages, spiral, sinuous, or straight, run along the surface of the membrane or plunge into its interior; it is the interweaving of their branches and their appendages that constitutes the tissue of the membrane, that is to say (Fig. 4, pl. 112) not only the ventral face of the electric lamina, but also the intermediate layer described by Ranvier. This fact can be verified, at the level of the tears in the membrane in the preparation, by changing the focus. We see that the entire thickness is made up of the tangled tissue of the branches of the nerve fiber covered with spore-bearing appendages.

This interweaving, in all directions, of the branches and their appendages and throughout the entire thickness of the membrane, obviously constitutes a lamina of great solidity and also *achieves an enormous multiplication of the volume and active surface of the end of the nerve.*

This device differs quite significantly from that described by Ranvier.

From these observations we must conclude that *the constitution of the membrane of the alveolus of the electric organ of the torpedo is identical to that of the sarcolemma of the primary muscle fiber; these two membranes are formed by the spreading of nerve fibers over a large surface.*

What constitutes the difference between the alveolus of the electric organ and the primary muscle fiber is the contents of the membranous sac formed by the nerve fiber endings.

For the primary muscle fiber, this content is, as we have seen, a bundle of parallel elementary mycelial fibers, each consisting of two elementary fibrils, one straight axial, the other external, wound in a spiral like a coiled spring.

For the alveolus of the electric organ, this content consists of a liquid into which branch nerve filaments that can only come from the myelinated fibers constituting the wall. These filaments have a characteristic, very special shape; they are very fine, *very long* filaments, wound in long, continuous spirals and laterally emitting fine appendages ending in a zymospore.

These filaments are very fragile and break during preparation. In fact, to prepare them, I have only obtained good results by crushing the contents of the cell between two slides that are slid over each other; they are fixed and dried by heat and impregnated with silver nitrate.

Fig. 1 contains, in its upper part, some fragments of these long spiral filaments similar to very long and thin coil springs.

A large number of these spiral filaments coil around themselves in the manner of a mycelial filament forming a perithecia, to form ovoid bodies. Some of these bodies are seen in Figures 6 and 7; the spiral coiling of the formative filament is clearly visible and, in some places, its elementary constitution, that is, its own spiral coiling. Scattered throughout the preparation are a multitude of fine, sinuous, branched filaments, which bear sinuous or spiral appendages ending in a zymospore. These filament fragments are the debris of the long spiral filaments and the lateral branches they emit (Fig. 6 and 7, pl. 112).

Due to the structural identity of the sarcolemma of the muscle fiber and the wall of the alveolus, it is likely that these two membranes fulfil a similar role in the two phenomena of motility or electrical discharge: that of triggering either muscle contraction or electrical discharge.

Having determined that the secondary muscle fiber is made up of two fibrils, one straight axial, the other wound in a spiral around it, it follows from this fact that, the

mechanical work being carried out by the tension of these coil springs, *the role of the sarcolemma is to cause and maintain this tension*; it fulfills this role, no doubt, by the modifications of its electrical tension (negative variation of the muscle in contraction).

Independently of this determination of the constitution of the active elements of the torpedo's electrical organ, it is therefore demonstrated that they are mycelial elements and that they belong to the specific organic mold, since they are brought into the organ by the nerve which controls its activity.

* * *

The mycelial nature of the torpedo's electric organ is attested by the following fact: If the substance of the organ, removed aseptically and ground, is cultivated on agar, the mold that constitutes it is obtained. I obtained this mold in the form of white colonies with long hairs, 2 to 3 millimeters or even more, affecting the *Aspergillus* gonidial type. As it aged, the culture took on a pale green tint, then yellow in the center and green at the periphery.

Examined, this mold presented particularities of great interest. It contained (Pl. 113):

1. Fruiting bodies identical to those carried by certain filaments of the membrane of the electric organ; these fruiting bodies of different shapes will be seen in figures 1 to 5, plate 113. In figure 4, a filament coils in a spiral to form a perithecia; on the blade of

the electric organ itself, similar perithecia are formed by the coiling of a fairly large branch; one will be seen in figure 3, plate 112.

2. We find, on the large mycelial filaments, the mode or arrangement of ramifications of the large nerve fibers of the electric organ called Wagner's bouquet. We see a mycelial branch of *Aspergillus* ramifying into a Wagner's bouquet in figure 3, plate 113 (left), and one in figure 7 (center); in the latter, the ramification of the mother fiber forms four branches emanating from the same point.

3. The large mycelial filaments of this *Aspergillus* have interannular constrictions, a central cylinder, and the moiré and pearly appearance of the myelin fibers and the organ itself.

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CHAPTER IV

NATURE AND CONSTITUTION OF PLANT TISSUES

Having determined that the animal organism is formed by the organization of a specific mold, it appeared that this must represent the ancestral form of each species at the origin of the appearance and development of living organisms on the globe. It also followed that, if molds are the ancestral forms of animals, they must probably also be those of plants; the culture of their tissues quickly confirmed this prediction. I was able to easily isolate organic molds from numerous plants and observe that each species is constituted by a particular mold, specifically different from that of neighboring plant species.

From this, it resulted again that a plant is constituted by *an organized* mold. The search for the elements of this mold, in a plant organism, demonstrates that current knowledge on the constitution of plant tissues does not correspond to their real organization.

The information I gathered during this research was obtained:

1. By a study of the leek leaf and the scales of the onion bulb.
2. By a study of the parenchyma of tubers (potato), roots (carrots), and fruits (apple, pear).
3. By a study of the parenchyma of the leaf of Althoea officinalis.
4. By a study of the constitution of the root.
5. By the study of the constitution of higher fungi (Hymenomycetes).

I. Study of the constitution of the tissue of the leek leaf
(Aillum porrum)

The tissues of the stem, bark, and leaf are described as being a mass of partitioned, polyhedral cells, grouped side by side, in which certain differentiated elements can be distinguished: woody-ligrous bundles, glands or secretory organs, epidermis, etc.

A thin cross-section of a plant gives the impression that this constitution of plant tissues actually exists. Figure 1, Plate 123, is a photograph of a portion of a thin section of the leek leaf. It does indeed give the impression of a parenchyma of polyhedral cells.

However, this constitution is not that of plant tissues. The leek leaf is the first plant organ that I examined to determine whether, in plants as in animals, the tissues contain traces of their mycelial origin.

Having made fairly thick cross-sections that I had stained with dahlia, my attention was immediately drawn to the particular appearance of the regions in which the tissues had been torn or pulled by the razor. Two of these regions, photographed, are shown in Figures 1 and 2, Plate 124. We note in these figures:

1. Firstly, the protruding parts A, B, of each figure, are large elements that ramify and whose branches diverge to spread out towards the epidermis. This ramification into several branches is not in doubt for element B, Figure 2.

2. That these elements are included in the meshes of an areolar tissue that constitutes their supporting structure and which is made up of anastomosing fibers limiting polygonal spaces.

3. Secondly, if we consider a distinct cellular element, such as A, Figure 2, whose membrane outline is visible, we see that the polygonal spaces do not correspond to the boundary of a cell. Example: cells B, Figure 1, and A, Figure 2, on which the supporting frame forms several polygonal spaces.

The polygonal spaces of the thin sections therefore do not represent the boundaries of the cells. Each polygonal space represents only one mesh of the supporting framework, the wall of a single cell being able, on a thin section, to correspond to two or three polygonal spaces; cells B, Figure 1, and A, Figure 2, are an example of this.

I have been able to observe, through numerous observations, the existence of the following arrangements in the parenchyma:

1. The supporting framework is formed by branched woody filaments, whose branches anastomose with each other to form the areoles; each branch is made up of a bundle of fibrils.

2. Most of the polygonal spaces are closed by a membrane, but not all are; some remain open and give passage to the mycelial tubes **(1)** along the path of which the cellular elements are formed.

3. The membrane that closes each polygonal space is formed by the spreading and ramification of fine mycelial branches emanating from the woody fiber constituting the perimeter of the polygonal space.

(1) *"Mycelial tubes" is used here and below as a synonym for large mycelial filaments.*

4. The entire support framework therefore constitutes, in reality, a series of rigid channels limited by a thin membrane and in which are contained the mycelial tubes of the organic mold and the cellular elements that they form.

These various arrangements can be observed by examining either cross-sections of the leaf stained with dahlia or impregnated with silver nitrate, or preparations obtained by scraping the parenchyma. The latter are made as follows: with the blade of a scalpel, scrape the surface of a leek leaf (lower part, 2 or 3 centimeters above the collar). Spread the scraping on a glass slide after adding one or two drops of water; dry hot and stain with dahlia after evaporation; then wash with hot water to remove excess dye.

The photographs in plates 125, 126, and 128 were taken from preparations made in this way. These preparations show the isolated elements of the parenchyma: mycelial elements, cellular elements, supporting carcass, membranes.

Mycelial elements

Figure 2, Plate 125, shows a group of mycelial tubes B, B, still with their supporting framework A, A, A, seen in the longitudinal direction.

Figures 3, 4, 5, and 6 contain mycelial tubes torn from their canals. Mycelial tube A, Figure 3, was torn out with two branches, B, C, which it emits.

Fig. 5 shows a mycelial tube of a special shape, identical to that of certain mycelial filaments of *Mucor* and *Aspergillus* or even *Penicilium*. It has a series of oval swellings A, B, C, D, E, which are poorly stained by dahlia, between which a basophilic and more stained constricted part H, H, H can be seen. This mycelial filament has a pedunculated spore F starting from a constriction H.

Fig. 1 shows a large mycelial filament of another form. This element, analogous to a myelinated nerve fiber of an animal, is identified by its transverse striations A, A, A, vestiges of the septation, and by its axis-cylinder B; this tube emitted lateral branches, some of which are still visible; it is an element of a libero-ligneous bundle. Fig. 6 contains a very long mycelial tube of *Mucor,* only part of the length of which has been photographed.

Supporting carcass and membrane

A fragment of this carcass is visible in Figure 6, Plate 125, at D; a fragment of the membrane torn off with it adheres to it. This element shows that the cell wall does not have, over its entire extent, the thickness that a thin section such as that in Fig.1, Plate

123 would indicate; on such a thin section, no distinction can be made between the thick upper part and the thinner lower part. When only the membrane exists in the section, the wall is thin; when it is the supporting filament that is seen, it is thick.

This known fact has been explained by a thickening of the membrane at certain points; the explanation is therefore inexact, the filament that forms the thickness not being a thickening of the membrane since it is this that forms it, as will be seen later.

Figs. 1 (gr. 1100) and 2 (gr. 610) of plate 126 represent another fragment of the supporting carcass to which adhere fragments of membrane torn off with it (gr.610); the filaments of the supporting carcass emit numerous lateral branches B, C, D, E, F, G, etc., which, by their ramifications spread out on a single plane, constitute the membrane which closes the polygonal space; the finest ramifications are covered with spirillary

and sporiferous appendages whose intricacy constitutes the membrane; figure 3 of plate 129 shows, on the other hand, a small fragment of the carcass, seen at a high magnification (1100 D), emitting fine fibers participating in the constitution of the membrane.

The constitution of the membrane by the supporting carcass is much more apparent in preparations impregnated with silver nitrate. Plate 127 shows parts of a transverse section of the leek leaf prepared in this way (Fig. 1, gr. 1100; figs. 2 and 3, gr. 610).

Following the edge of the supporting carcass constituting mesh A, figure 1, we see numerous branches branching off into fine twigs which bear a multitude of straight or spiral spore-bearing appendages. On examining the points where fragments of membrane are well preserved (A, fig. 2), we see that they have the appearance of a trellis identical to that of a basket, that is to say, formed by numerous small parallel branches crossed in two perpendicular directions.

There are also small isolated masses on the membranes from which filaments radiate in all directions and bear sporiferous and spiral appendages (C, B, fig. 1, pl. 127). *These masses, examined under a magnifying glass, appear to be rigorously similar to the neuro-glial cells of the brain, spinal cord, and nerves.* They are in fact of the same nature; in animals as in plants, they are small germinative masses in the process of development.

The woody filament that constitutes the supporting carcass is not made up of a single fiber, but of a bundle of fibrils; its nature is identical to that of the spiral fiber of the so-called spiral vessels; there is a certain identity between these two elements because the fibers of the supporting carcass of the cells emanate from the spiral fibers of the vessels.

Fig. 3, Plate 126, contains a fragment A of the spiral fiber of a vessel. This spiral is considered a thickening of the wall; it is actually constituted by a bundle of fibrils, wound in a spiral, the turns of which are joined together by a thin membrane formed by the fiber itself; following the filament A, Fig. 3, Plate 126, we can distinguish the thin lateral branches which it emits to constitute the membrane.

The spiral filament of the vessels is therefore a large mycelial fiber constituting the supporting carcass of the canal and at the same time supplying, by lateral ramifications, the supporting fibers of the parenchyma.

The emission of the latter by the spiral vessels is clearly visible in Fig. 2, Plate 123.

As for the relationships that exist between this spiral fiber and the elements of the supporting carcass of the parenchyma cells, they can be established without difficulty by examining transverse sections of the leaf impregnated with silver nitrate, such as that represented by a photograph in Figure 3, Plate 123.

This figure shows a group of spiral vessels from which emanate the fibers constituting the supporting carcass of the subepidermal parenchyma (examine with a magnifying glass). The same demonstration is repeated in Figure 1, Plate 130.

The filaments constituting the supporting carcass of the sub-epidermal parenchyma send out branches that penetrate between the epidermal cells and form, above them and on the surface of the leaf, a fairly thick network of highly ramified branches covered with spore-bearing appendages that constitutes the cuticle (B, D, fig. 1, pl. 129).

This network sends out, inwardly, between the epidermal cells, ramified branches (C, fig. 2, pl. 129) having a configuration similar to that of clustered glands.

In the layer of epidermal cells, we notice certain elements that have the structure of a spiral vessel (A, fig. 1, pl. 129) and whose canal extends to the surface of the leaf. I have not been able to determine whether or not they communicate with the exterior.

Cellular elements of the parenchyma

In preparations of the leaf scraping, numerous spherical or oval elements are observed to which a portion of the *Mucor* mycelial tube that formed them is still adherent (Figs. 1, 2, 3, 4, pl. 128). Some (Fig. 3) are mycelial dilations of a *Mucor*-type filament; others are terminal formations (Figs. 1, 2, 4).

The element represented in Figure 3 is a mycelial dilation, because the formative filament remains visible at its entrance and exit; the element contained in Fig. 1 is a terminal formation.

On the other hand, vestiges of the supporting carcass can be seen on these elements. By carefully examining the sub-epidermal parenchyma on the right of Fig. 2, Plate 123, with a magnifying glass, one will distinguish, *under the supporting carcass (frame)*, identical formations as well as the filament that forms them and those that emanate from it. *Filaments will be seen running through the meshes of the areolar tissue.*

Examination of the elements contained in figures 1, 2, 3, combined with a magnifying glass examination of the section represented in figures 3, plate 123, demonstrates:

1. *That cellular unity or independence does not exist:* each element of the parenchyma, considered until now as a cell, is formed by a mycelial filament on which it depends and which, itself, is not independent, because it emanates from another filament larger than itself; *by going back to the origin of all these elements, we would arrive at the germinative mass that formed them. The cell is not independent, because if we were to cut the mycelial filament that forms it, it would perish.*

2. That there is no correspondence between the polygonal meshes that a thin transverse section presents and the cellular elements, a fact already explained previously.

The results of this series of observations can be summarized as follows:

1. The tissue of the leek leaf is made up of the vegetation of a mold whose largest and longest mycelial elements are grouped into bundles of parallel fibers, which are the libero-ligneous bundles.

2. The cellular elements of the parenchyma are dilations formed along the path or at the end of numerous mycelial ramifications emitted by the fibers of the libero-ligneous bundles.

3. Around the cellular elements of the parenchyma exists a supporting structure formed by woody filaments; these constitute, through their anastomoses, polygonal spaces that limit polyhedral areoles in which the mycelial filaments or their formations travel. These areoles are not completely closed; they constitute, in short, canals in which the mycelial elements vegetate.

4. The woody fibers that constitute the supporting structure emanate from the spiral vessels.

5. There is no correspondence between the polygonal spaces that appear in thin sections of plant tissue and the cellular elements or dilations formed along the path of the mycelial filaments. A polygonal space does not represent a cell.

6. The cellular elements or mycelial dilations have no individuality or independence; their life and nutrition depend on the mycelial filament that forms them and on the distant sclerotium that gave rise to it.

Constitution of the onion bulb scale.

Microscopic examination of thin sections reveals an organization in the onion bulb scale identical to that of the leek leaf. A supporting structure can be seen (Fig. 2, pl. 130) in the mesh of which mycelial branches swollen into balls (B, E, F) travel along their path and at their ends. The supporting structure clearly emanates from the libero-ligneous bundles. These balls are, moreover, very clear on the torn edge of certain preparations (Fig. 3 and 4, pl. 131); on some of them, the supporting structure can be seen. If, instead of coloring a cross-section with dahlia, it is heavily impregnated with silver nitrate, a multitude of details appear which were invisible in the colored preparations.

In certain areoles of the supporting frame, which still contain their ball cut transversely or intact, one can distinguish the structural details of the interior of the latter (B, C, fig. 1, pl. 131); one sees that this ball is made up of coiled, twisted, branched filaments covered with sporiferous appendages; certain filaments are terminated by larger spores which are, in certain cells, grains of chlorophyll and, in the deep cells, colorless leucites. In the center of the ball is often found a rounded mass, the nucleus, made up of a group of coiled filaments; Several of these nuclei are visible in Fig. 2, Plate 131.

Other interesting details emerge in the sections impregnated with silver nitrate. In those contained in Plate 132, it is clearly visible that the fibers constituting the supporting carcass emanate from the libero-ligneous bundles and, in particular, from the spiral vessels.

In Fig. 3, representing a small libero-ligneous bundle, it is clearly visible (examined under a magnifying glass) starting from it the fibers constituting the supporting carcass and their intricacy at the angles of the areoles; some fibers, larger than the others, branch out after a certain distance.

In Fig. 1, Plate 132, larger mycelial filaments forming the balls (B, C) described above can be seen; these filaments and the balls they form also emanate clearly from the bundles.

II. Constituent elements of the carrot parenchyma.

To examine these elements, a carrot is cut longitudinally through the middle, the cross-sectional surface of one of the parts carefully scraped with a scalpel blade, and the resulting pulp placed on a heat-dried slide, then stained with dahlia.

These preparations contain:

1. Long mycelial filaments of various shapes emitting lateral branches (Fig. 2, 3, pl. 133), and often bearing a very apparent partitioning (Fig. 3). Others have an axial cylinder of basophilic protoplasm, like the mycelial filaments of *Mucor* and *Aspergillus* (Fig. 1, 2, 3, 4, pl. 134). The ends of the ramifications form a fine areolar tissue whose filaments are covered with sessile or short-pedunculated zymospores (Fig. 5, pl. 134).

2. Large spherical or oval balls on the surface of which the supporting carcass, torn off with them, is still visible (Fig. 6, 7, 11, pl. 134).

Some balls are still connected by their pedicle to another mycelial dilation that gave rise to them (Fig. 9, pl. 134).

The external membrane of these balls is formed, like animal membranes (sarcolemma for example), by the division of a mycelial branch, the spreading and ramification of its branches on a single plane (Fig. 10, 12); the finest ramifications form a fine areolar tissue whose tortuous branches are covered with spiral or straight appendages ending in zymospores (Fig. 8, pl. 134).

If we examine a thin longitudinal section of the carrot, stained with dahlia, we see that the parenchyma is made up of a multitude of polygonal sacs, packed against each other and held together by a solid, fibrous mycelial framework, which constitutes the supporting structure of the tissue (Fig. 1, pl. 133). On the torn edges of the section, we can more clearly distinguish the shape of the sacs and the independence of the supporting structure.

Constituent elements of the parenchyma of an apple.

In preparations made as indicated above, we observe a constitution of the parenchyma identical to the previous one. We note:

1. Mycelial filaments, partitioned and branched (Fig. 2, pl. 135). Some of these filaments have a cylinder-axis of basophilic protoplasm (Fig. 1), like the filaments of *Mucor,* and emit short branches on which are born the large balls or spherical sacs that are considered as the cells of the parenchyma (Fig. 5).

2. Spherical or ovoid balls or sacs; the transparent wall of some of them allows one to perceive the twisted and coiled mycelial filaments which constitute the organization of the cell as well as their fine ramifications constituting an internal areolar tissue loaded with zymospores; larger spores are formed by the large filaments (Fig. 3, 4, pl. 135); the fine ramifications of all the mycelial branches, either in the membranes, or in the cells, or on their exterior, end in a similar areolar tissue, covered with appendages bearing zymospores (Fig. 6, 7, pl. 135).

The observations made so far show that the plant parenchyma is essentially made up of:

1. By mycelial elements dilated into a spherical or oval ball, a single filament being able to carry a series of these dilations; these are the elements that have been considered as the cells of the parenchyma. These dilations or cells are not autonomous, a series of them being formed by a mycelial filament which, itself, depends on a larger branch from which it emanates.

2. By a fibrous supporting carcass whose elements form an areolar network in the meshes of which the mycelial elements develop.

3. By spiral vessels; their spiral fiber is independent and is constituted by a mycelial filament (or a bundle of fibrils) giving rise laterally to the fibers of the supporting carcass.

4. By partitioned, longitudinal mycelial filaments, emitting numerous lateral ramifications which carry the dilations which are considered to be parenchyma cells.

This knowledge has been acquired by various means, either by the study of dissociated elements, or by the study of transverse or longitudinal sections; if the anatomical conformation which I have just described has not been seen until now, it is because the process used for studies of botanical histology, the examination of thin sections, is incapable of clearly detecting the relation- ships which exist between the elements.

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However, it is desirable to be able to ascertain these relationships with certainty, particularly to establish that cellular independence does not exist and that *every cell, considered until now as independent, lives by the mycelial filament which forms it and which depends on the distant sclerotium from which it emanates.*

I was able to clearly establish, in the following study, that the *elements of a plant parenchyma are formed by mycelial ramifications which emanate from the elements of the libero-ligneous bundles and by the terminal ramifications of these elements.*

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III. Study of the relationships that exist between the libero-ligneous bundles and the elements of the parenchyma, in the leaf of Althea officinalis.

To carry out this study, the elements of a libero-ligneous bundle must be made visible so as to be able to follow their path and observe the relationships they present with the cells of the parenchyma; to achieve this condition, a fairly large flap of epidermis must be torn off with fine forceps from the underside of a leaf, that of *Althea officinalis*, for example; this flap is torn off along a vein so as to uncover it at the same time as the parenchyma; it is enough to tear off a flap 5 to 6 millimeters on each side to have a wide area for examination, but the operation is difficult; it is a game of patience which requires an hour or two to be successfully completed. The uncovered area is cut off with a scalpel and placed on a glass slide, along with the removed flap, the bare parenchyma on top; a drop of water is added and the slide is covered.

As will be seen from the photographs attached to this presentation, the examination of the preparation is quite easy, provided that one refrains *from any staining*; indeed, the photographs in plates 136, 137 were obtained on an unstained preparation. Photography is more difficult to achieve than direct examination; the light projected onto the preparation causes an intense chemical reaction in the living plant tissue and very quickly causes a cloudiness to appear which obscures the preparation; this cloudiness is visible on the left of photographs 1, 2, 3, plate 137.

In these preparations, the study of the parenchyma cells is facilitated by the fact that they are arranged in chains of three to six separated from each other by the airbearing lacunae; their clear contours and the transparency of their membrane allow the study of their reciprocal relationships.

The possibility of following an element at various depths, by varying the focus of the microscope, makes it possible to see exactly its starting point and its arrival point; also, direct examination by eye makes it possible to give a more precise demonstration than the examination of a photograph, the latter being clear only on one plane.

The original photographs of plate 136 (Figs. 1 and 3) are made at the level of a small rib of which at least two elements presented the punctuations of the sieve tubes; Figs. 2, 4, 5, are enlargements of the previous ones (2 D). By direct examination of the photographed region, I clearly saw:

1. That the punctuations of the sieve tubes, seen from the inside are, at least for a large number, holes corresponding to the canal of a mycelial filament, a lateral branch emitted by the sieve tube.

2. That the filament thus emitted enters, after a short journey, into a cell of the parenchyma and that it is this which, by ramifications, constitutes the latter.

3. That the cell formed emits one or more other mycelial filaments, generally very short, which in turn form another cell attached to the first and following it in a chain; sometimes, a mycelial tube emitted by a cell will constitute another much further away.

4. That the tortuous and coiled filaments in the cells form *green* spores, which are clearly seen pedunculated and adherent to the mycelium, and which are the grains of chlorophyll.

5. That the tubes, sieve-like or not, which constitute the vein, are mycelial filaments of the organic mold, and that the elements of the parenchyma are mycelial dilations in a string born on the lateral branches which they emit.

By direct examination, it is very easy to see and follow the mycelial branches which, starting from the septate tubes of the vein, go to the cells. By examining Figures 3 and 5, Plate 136, with a strong magnifying glass, we will find a certain number of these ramifications quite clearly visible at A, B, C, D, E, F, G, H, I, U, V.

In Figure 5, Plate 136, we can follow a lateral ramification B (which has been reinforced by a dotted line and whose orifice in the mycelial tube of the vein is marked by the shade A), to the cell J which it forms; this emits several new branches, one of which forms the cell K which, itself, gives rise to the cells L, Q and T, the cell Q being connected to K by the filament R; the cell N is formed by a filament coming from the cell O.

The mycelial tube that connects two cells is formed by a spiral filament: a filament of this kind, P, is clearly visible between the two cells N, O, which it connects. Thus appears the connection that exists between the cells that constitute the parenchyma of the leaf; these cells have no individuality; they are elements formed by mycelial filaments emitted by the long partitioned tubes which constitute the veins, that is to say by the elements of the libero-ligneous bundles.

Let us note, here, that certain normal elements of the parenchyma (A, B, C, fig. 4) have a particular form which we will recall later; we will indeed note, later, that the teliospores of *Puccinia malvacearum*, which were taken for spores of a parasitic fungus, are elements belonging to the plant, but deviated in their form; the teliospores of the pustules of *Puccinia* frequently have the form of the elements A, B, C, figure 5, notably before their complete development.

The parenchyma of a normal leaf of wheat, barley, or oat (a young leaf of a seed that has germinated for eight days) also contains elements identical to the teliospores of

the puccinia of these grasses; on the other hand, the rounded cells of the parenchyma are the elements which, vegetating abnormally in the new conditions where they have lost their chlorophyll, have been taken for uredospores.

The connection which has just been explained between the various elements of the *Althoea* leaf can be established in an equally convincing manner by following the partitioned and parallel mycelial tubes which constitute the vein, to determine what becomes of them at their termination.

If we follow a fairly large rib, made up of a large bundle of these elements, we notice that those on the periphery end abruptly, one after the other, branching out in a particular way to form elements of the parenchyma. Figures 1, 2, 3, of plate 137, represent a region where three fibers A, B, C, of a rib branch out; these three figures are photographs taken with different focusing.

If we follow only the two fibers B and C, Fig. 1, we see that the branching occurs in the following manner:

1. Fiber B, figure 1, ends with a last partitioned element D which emits three others, E, F, G, by branching; we can vaguely guess the branching of elements E and F; as for element G, it emits three branches L, M, N. This region is veiled by a fog which forms quickly and is due to the reaction of the chlorophyll elements to the light of the electric arc.

2. Fiber C, figure 1, ends with the partitioned article H which emits, by branching, three cells, I, J, K, which already have the form of the normal elements of the aeriferous parenchyma. The birth of these three elements on article H is most clear in figures 1 and 2.

They themselves form four new elements, P, Q, R, S, visible more clearly in figure 3 (F, H, I, J); these in turn form new ones, *by the emission of several small mycelial tubes, clearly visible at the upper part of the elements O, P, figure 1*. If we examine with a magnifying glass the region which is above the cells O, P, Q, R, figure 1, we see several of these fairly long tubes, one of which T ends at the cell which it forms.

If we now examine the fibres which constitute the rib, we see that they do not differ from the large mycelial filaments of moulds of the *Mucor* and *Aspergillus* type; *they present the same partitioning features, possess like them interannular constrictions and a very visible cylinder-axis* (A, B, C, fig. 2; A, B, C, fig. 3, pl. 137).

Remembering that the myelinated nerve fiber, through its ramification, entirely constitutes the laminae of the torpedo's electrical organ, we must conclude that certain fibers of the veins of the leaf of plants, mycelial filaments of the *Mucor* or *Aspergillus* type like

the myelinated fibers, are morphologically similar to the latter and are assimilable to them.

They must play, with respect to the elements of the plant parenchyma, the trophic role that nerve fibers play with respect to the elements of the tissues or organs of the animal.
The parenchyma of the leaf of plants is therefore constituted by the lateral and terminal ramifications of the mycelial fibers constituting the libero-ligneous bundles (notably sieve tubes); these ramifications form, along their path, globular elements which are the cells of the parenchyma.

Plant leaves are affected by diseases characterized by the presence of pustules or excoriations, the formation of which has been attributed to parasitic fungi. The quince leaf, for example, presents excoriations produced, it is believed, by the vegetation of a parasite: *Stromatinia cydoniæ*, whose spores, accumulated under the epidermis, lift and tear it. Figure 4, plate 138, represents, according to Delacroix and Maublanc, a gonidial fructification of *Stromatinia*. It can be seen in this figure that the terminal branching mode of the parasite's mycelial filaments is identical to the terminal branching mode of the mycelial filaments of the veins.

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In addition, the gonidia (enlarged in the lower part of the figure) *have an identical constitution to the elements of the leaf parenchyma and are joined together* by a filament (*disjunctor,* inaccurately represented in the figure) identical to that which connects the cells of the parenchyma together (filament P, fig. 5, pl. 136).

This identity comes from the fact that the lesion is not produced by a parasite; the mycelial filaments which branch out under the epidermis and form abnormal elements there which have been taken for gonidia are not those of a parasite, but *those of the libero-ligneous bundles of the leaf itself* which, *under the influence of unfavorable causes*, vegetate in an abnormal form.

Proof of this is easy to provide by cultivating the gonidia on sugar agar and, at the same time, the healthy tissue of the bark or of unaffected leaves*. In both cases, the same gonidial forms of the organic mold of the quince tree appear.*

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IV. Constitution and Nature of the Root.

For this study, wheat or oat grains are germinated in fine, moist sterile sand. When the seedling is 10 to 15 centimeters tall, the mass of sand containing the roots of a seedling is removed with a spoon and carefully placed in a vase full of water; the sand falls out without noticeably damaging the roots. The rootlets are cut one centimeter from their

tips; they are placed in a weak dahlia solution, then in pure water; they are then placed on a glass slide on which five or six drops of water have been placed to allow the rooting hairs to spread. Plate 138 contains photographs of wheat rootlets prepared in this manner. In such preparations, we observe:

1. That the young root is formed by parallel mycelial filaments grouped in bundles which, towards the end of the rootlets, perforate the external membrane to form the root hairs (Fig. 1, 2, 3, pl. 138).

2. That the root hairs thus formed are mycelial filaments having the characteristics of *Mucor*-type vegetation (Fig. 6).

3. That some of these filaments form at their ends the fructifications characteristic of the *Mucor* type. Two filaments, A, B, bear a fructification in Figure 2. Figure 3 shows another filament ending in a fructification whose spores have fallen, but still having its columella in place.

4. That between the root hairs, and all around the rootlet, there is an abundant vegetation of fine mycelial filaments emitting zymospores laterally (Fig. 5, pl. 138). Some of these filaments appear to emanate from the root hairs, but most emanate from germinative masses which give rise to them, or from spores of the germinated fructifications of *Mucor*.

5. That the tips of certain root hairs, rich in basophilic protoplasm, segment to release large articles having the same diameter as the mycelial filament and a length about twice as great; these articles, *which are in reality enormous bacteria*, become germinative masses and emit fine mycelial filaments.

The root is therefore constituted, like the leaf, like a tuber, by organized elements of a growing mold.

The root hairs, intended for absorption, are constituted by mycelial filaments which, emerging from the root, insinuate themselves between the fine particles of the soil.

The observations we have just made have a broader scope and make us aware of a new, unknown function of capital importance in the nutrition of plants.

This new function is revealed:

1. By the fact that the mycelial filaments which constitute the rooting hairs emit, at their free end, that is to say outside and in the earth, fructifications of the *Mucor* type, or, by segmentation, articles which function as germinative masses.

2. By the fact that these germinative masses or the spores of these fructifications, once emitted into the earth, evolve and constitute around the rootlets an independent mycelial vegetation which forms zymospores, spores and bacterial elements.

Thus, the mold constituting the plant rejects outside, into the earth, a large quantity of spores or other elements in order to develop the mycelial vegetation whose existence is observed around the rootlets and with the aim of forming zymospores. This is therefore a function similar to that which consists, for animals, in expelling into the stomach or intestine, in the form of digestive ferments, the zymospores with special activity which are formed in the digestive glands.

Since plant foods spread throughout the earth cannot be absorbed without undergoing chemical transformation, the role of the external fructification, in the earth, of the mycelial filaments constituting the root hairs of the rootlets, becomes clear; this fructification is the act of producing a digestive or transformative juice.

V. Constitution of Higher Fungi.

Since the constitution of the parenchyma is identical in all hymenomycetes, there is no need to choose among them to study it; the species used for my description is *Collybia velutypes*. A piece of the stem is finely dissociated on a glass slide, in a drop of water; the preparation is dried while hot, stained with dahlia, washed, and dried again.

Examination of such a preparation shows that the fungus's stem has a very simple constitution; it is formed of parallel mycelial tubes identical to the *Mucor*-type filaments of molds. Bundles of these tubes are photographed in Figures 1, 2, and 4, Plate 139. They show the partitioning lines and an axial cylinder with constrictions at the level of these. One of these tubes, showing its axial cylinder, a partitioning line, and a constriction, is isolated in Figure 5. Besides these *Mucor*-type fibers, there are other smaller fibers (A, Fig. 4), generally not having an apparent axial cylinder, with very rare partitioning lines or not partitioned. These fibers differ from the previous ones by the same characteristics that differentiate Remak fibers from myelinated fibers in nerves. These fine fibers emanate either from *Mucor*-type fibers or from germinative masses included between them.

At certain points along their path, some fibers exhibit interesting spherical, oval, or spindle-shaped dilations, either the axis-cylinder alone or the entire fiber (A, B, fig. 1, pl. 139); these dilations follow one another in a chain and are separated by a constriction rich in basophilic protoplasm.

An isolated *Mucor*-type tube shows, in figure 6:

1. Lanterman's network.
2. The axis-cylinder and a constriction.
3. A multitude of small spiral spore-bearing appendages on the surface of the mycelial tube.
4. The spores and zymospores that exist in large numbers around the mycelial tube (also visible in figures 3 and 5).

The stem of a hymenomycete fungus is therefore made up of a bundle of parallel mycelial tubes that then spread out like an umbrella to form the cap. ***Mushrooms therefore constitute the first organized forms of molds.***

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Each species of fungus is formed by a specific moiety; I have isolated and cultivated organic molds from many species of fungus; some are represented in plates 326 to 329; the gonidian forms obtained are mainly of the *Penicilium, Aspergillus, Mucor, Verticilium, Monilia, Acremonium*, etc. types.

Knowledge of the nature of mushrooms, cultivation, and the study of the properties of the organic mold that constitutes them demonstrate that *they are not toxic but pathogenic*; the very dangerous substance contained in certain amanitas is constituted by the zymospores of the organic mold; the power of these zymospores is not destroyed at 100°.

* * *

The study of plant tissues therefore demonstrates that they are made up of an organized mold.

Plants are made up of bundles of mycelial elements (woody-woody bundles) which emit around them a multitude of lateral branches forming the elements of the parenchyma (wood, bark, leaves) along their path.

Knowledge of the nature and constitution of plant organisms will allow us to establish, later, that the diseases that affect them are autogenous and not due to parasitic elements; they result from an abnormal transformation of the specific organic mold placed in defective nutritional conditions.

The results of the study of some of these diseases have unforeseen and important consequences because they provide us with a first precise notion of *the evolution of organized beings, that is, of the organization of molds* and the formation of species of organized beings. I present these facts here because they relate to *the nature and origin of fungal species.*

The disease called grey rot of grapes has been attributed to a parasite called *Sclerotinia fuckeliana*. We know that this parasite occurs in two different forms: on grapes, it has

the form of a grey mold, *Botrytis cinerea*; this mold forms, by rolling up its filaments, black balls called sclerotia. These, when allowed to germinate in damp sand, develop into a fungus already clearly organized, a *Peziza.*

This is an important and well-known fact showing that a mold can give rise to an organized fungus, and whose importance, until now, has not been fully appreciated in relation to the evolution of organized beings.

By studying this disease, I was able to establish that *Botrytis cinerea,* which develops on grapes, is not a parasite, but a gonidial form of the specific mold of the vine. *Peziza Fuckeliana*, the second form of the parasite, is therefore also a *vegetative form of the organic mold of the vine.*

Bringing this fact together with others already explained previously, we now know:

1. That when a plant individual, or only one of its parts, dies, it is only its organization that disappears. The living matter or organic mold that constituted the individual does not die; it only changes form and passes into the state of wandering, unorganized mold.

2. That mold that has lost its organization and survives, vegetating in all the environments to which it is transported, forms sclerotia, which are a mass of mycelium curled up for a special purpose: the formation of large spores, which are, in short, eggs. The sclerotium is therefore an *already organized* formation that immediately reappears in the mold.

3. That this sclerotium will reproduce the same mold if it is sown on sand, but that, if it is placed in other conditions, it will form a new species with a very clear organization and much more complicated than that of the sclerotium: it is enough to bury the sclerotium in sand and it will germinate into a mushroom, the *Peziza Fuckeliana.*

Thus, when a plant, the vine, for example, dies, it is only its organization that disappears; its living organic matter leaves the organism and develops freely, without spatial limits for its development, *in the form of an unorganized mold*, but which, *if it finds a favorable condition, immediately reorganizes itself and forms a new organized being completely different from the first, a fungus.*

In the same way, when dying, the animal organism releases the organic mold that constituted it and which also vegetates in all suitable external environments, on earth for example. The mold released forms sclerotia, like mold from a plant organism. These sclerotia can therefore give rise to a fungus.

Thus, appears the origin of fungi. *This is indeed the first stage in the organization of living matter.* They come (at least some species) from the *perhaps recent and continuous* organization of the wandering organic mold of animal and plant species released by the death of individuals.

It will probably soon be possible to determine from which plants or animals the most well-known species of fungi originate, by comparing the gonidial forms of the organic mold of these species with those of the organic mold of plants and animals. I have already isolated a large number of gonidial forms of the organic mold of the most widespread fungi; I also have that of a certain number of plants, but still insufficient.

By this comparison we will be able to know, for example, if *Cortinarius largus* is almost always found preferably under beeches because it is a *reorganized* form of beech mold released onto the ground, around the trunk of the tree, by the leaves and fruits that have fallen from it.

* * * * *

CHAPTER V

We have now reached the end of the investigations aimed at determining the nature of the anatomical elements of the tissues of living beings.

The results obtained profoundly modify current conceptions on the histological constitution of animal and plant tissues and on the physiological phenomena of which they are the seat.

A living being is constituted by an organized mold identical in all individuals of the same species. This mold is the ancestral form of the species.

In the animal organism, blood is the culture medium of organic mold. Red blood cells are elements of the mold that take external oxygen to carry it to all the points where it vegetates. White blood cells are agglomeration masses or germinative masses intended to form new elements.

Zymospores are fine spores endowed with fermentative power when they are free; they are born in the blood on mycelial filaments emitted by spores or by other elements; they are also born on mycelial filaments of the mold constituting the organs and possess, in these, particular properties.

The anatomical elements of the organs, liver, various glands, etc., discharge into the blood differentiated zymospores which are particular to them.

The digestive glands discharge into the intestine their differentiated zymospores which constitute the digestive ferments, pepsin, trypsin, etc.

This new knowledge was most suggestive; it was well apparent that they shed a bright light on many phenomena that had remained obscure until now and whose determinism was impossible without this knowledge. How can we clarify the mechanism of anaphylaxis without knowing that an anaphylactizing substance contains the *living elements* of the organic mold of the being from which it comes, and that it is the development of this mold, in the prepared animal, which constitutes the state of sensitization and anaphylaxis?

How can we clarify the meaning and mechanism of blood coagulation without knowing that this liquid is a perfected culture medium whose elements endowed with chemical activity are the zymospores of the mold that vegetates there?

How can we know exactly how a digestive gland works without knowing that the active elements of the juice it secretes are the zymospores of its cells?

It was not possible to arrive at knowledge of the nature and mechanism of the phenomenon called immunity without knowing the nature of the animal organism and the intimate phenomena of mold development.

How can one know the nature of tuberculosis, cancer, and a large number of diseases without knowing that the organism is constituted by a mold which, under unfavorable conditions, can change its vegetative form and thereby constitute either tumors or autogenous and spontaneous bacterial cultures?

The necessary complement to the investigations that resulted in the determination of the nature of the animal organism was therefore a new series of studies aimed at the determinism of the physiological phenomena of which organic mold is the active agent.

In this work, only a few of them are mentioned. A selection has been made from among them, with a view to grouping together the knowledge necessary to achieve a result of practical utility, the urgency of which is evident: knowledge of immunity, its nature and its mechanism; the determination of the modification brought about in an organism by vaccination or by an immunizing disease.

We already possess elements of great importance for this determination; these are:

1. Knowledge of the nature of bacteria and the intimate phenomena of the development of molds in vitro and in the animal organism.

2. Knowledge of the nature of normal white blood cells formed by organic mold.

3. Knowledge of the formation, in the animal organism, of heterogeneous white blood cells by external pathogenic and infective agents.

The new studies that complete the determinations necessary to demonstrate the nature and mechanism of immunity relate to:

1. The nature of ferments of animal and plant origin.
2. The nature of bacterial toxins and antitoxins.
3. The physiological significance and mechanism of blood coagulation.
4. The mechanism of specific hemolysis and natural hemolysis by a heterogeneous serum.
5. The mechanism of agglutination.
6. The mechanism of precipitation.
7. The mechanism of the complement fixation reaction.
8. The mechanism of anaphylaxis.

9. The body's means of defense.

We will successively present the study of these various questions.

I. Nature of Ferments of Animal or Vegetable Origin.

Ferments of animal or vegetable origin, isolated in the dry state or kept in solution, are made up of various elements of the organic mold of the being producing the ferment, or of a special, differentiated part of this being. The solution of a ferment spread on a slide, dried while hot, stained with dahlia, and washed, contains:

1. An innumerable quantity of basophilic zymospores, granules of 0.3 to 0.5 μ.

2. Other zymospores of the same size and especially many larger, hyaline ones; the most numerous are about 0.5 to 1 μ. Others are 1 to 2 and even larger.

3. Bacillary elements in certain cases.

4. Numerous mycelial elements, mainly very fine, sinuous, often branched filaments bearing basophilic zymospores and spores of 0 μ 5 μ 1 μ.

5. Agglomeration masses of all sizes; some have their agglomerated elements (spores and zymospores) still distinct; in others these elements are fused and the mass (which is basophilic to varying degrees depending on the quantity of basophilic elements it has incorporated) sometimes presents, depending on the degree of its evolution, the beginnings of the broken mycelial filaments it has already emitted.

The active element of the ferment is the zymospore of the organic mold.

The active elements of the ferment are the zymospores. If a ferment solution is centrifuged, it is freed from the heaviest coarse elements and a large number of zymospores that fall to the bottom of the tube. The solution remains cloudy because it retains an innumerable quantity of zymospores that remain in suspension. The ferment solution centrifuged in this way retains its activity. If it is treated with a precipitate, such as tricalcium phosphate, if necessary several times in a row, and then centrifuged, it completely loses its activity; the solution thus treated, examined under a microscope, no longer contains zymospores. If the precipitate is examined, it is seen that it has carried them away (at least most of them). The centrifuged liquid being completely decanted, the precipitate of tricalcium phosphate being inoculated into broth, the zymospores are resuspended and develop a culture of the ferment.

The elements of the ferment are living mold elements.

This fact is demonstrated later in the study of the development of pepsin and diastase in germinating barley.

It is also demonstrated directly by inoculating the slightly moistened powder of the ferment onto agar; the mold from which it originates develops in three to six days in one or more of its gonidial forms. The experimenter who wants to convince himself that the mold obtained does not come from a banal contamination will inoculate in the same way numerous samples of ferments from various sources or from different harvests. He will always obtain a constant mold. *A contamination is inconstant and variable.*

He will also be able to verify that this mold is one of the gonidial forms of the organic mold of the being from which the ferment emanates.

Heating to 58° or a higher temperature destroys fermentative activity because it suppresses the vegetative activity of the ferment.

This is verified by dividing a ferment emulsion into two portions, heating one to 58° for thirty minutes, and then inoculating the same amount of each into a nutrient solution, even a simple glycose or glycose and maltose solution (5% each). The unheated emulsion develops new elements and colonies in two or three days; the heated emulsion develops nothing (or nothing that is noticeably apparent).

However, this cessation of vegetation is not definitive; the zymospores are not killed at 58°. By inoculating them in an appropriate medium, fermentative activity is seen to reappear little by little after several days, as soon as the mold vegetation has sufficiently developed.

This is easily demonstrated with lab-ferment, for example; the ferment heated for 30 minutes at 58°C is inactive. Inoculated in a sterilized 1% lactose solution, its activity gradually reappears after three to six days; it regains the power to coagulate milk.

The activity of organic ferments is theoretically inexhaustible, because they vegetate and multiply in the liquids where their chemical activity is manifested, these liquids constituting a favorable culture medium for them.

This multiplication and proliferation is demonstrated in the two examples cited below.

Here the facts explained in the chapter *"Development of Bacterial Cultures in a Liquid Medium"* come into play. In the same non-renewed liquid, the development of the ferment follows that of all cultures, *even if the liquid contains a practically inexhaustible quantity of transformable substance;* the culture clumps together and settles after a certain time, and fermentation stops.

A minimal quantity of the culture, transferred to a new nutrient liquid, reproduces the ferment just as a microbial culture, inoculated after its development has stopped, reproduces a new microbial proliferation.

It is for this reason that the action proportional to mass does not exist for a ferment acting for a relatively long duration and only occurs, to a certain extent, for the ferment exerting its action in a short space, the lab-ferment for example.

Over a long period, the quantity of zymospores tends to equalize quickly in solutions containing different quantities of ferment. In the space of 30 to 60 minutes, the proportion of ferment, that is, of zymospores, exerts an action, because they do not have time to reproduce to tend towards the equalization of the quantities involved.

Culture and Development of Ferments

I studied the development of various ferments. Plate 114 shows the phases of development of the diastase of germinated barley; Plate 115 that of barley pepsin.

I also carried out the transformation into hyphomycete of brewer's yeast (plate 306) and of a yeast used by Parisian bakers to make bread rise. The *Penicilium* form of brewer's yeast, cultivated on sugar agar, transforms sugar into alcohol like yeast.

Finally, further on, we will find another example applying to the development of the ferment that causes blood coagulation or the coagulation of fibrinogen solutions in vitro: the serozyme of horse blood.

Barley diastase

The diastase emulsified in water and immediately examined under a microscope contains:

1. A multitude of spores and zymospores, a large cluster of which is visible on the left of Fig. 1, Plate 114. Beside them, in the same figure, we see another group of spores, some of which have retained the Nycelian filament carrying the zymospores that they emitted into the medium from which the ferment is extracted.

2. Bacillary elements (Fig. 2).

3. Agglomeration masses or germinative masses formed in the medium from which the ferment comes (Figs. 2 and 3).

The diastase being inoculated in broth preserved at 18-20°, it is observed, after 48 h, that most of the spores have germinated and emitted a short, sinuous mycelial filament, bearing small lateral appendages ending in zymospores (Fig. 4). In addition, the elements have united to form agglomeration masses or germinative masses. In Figure 3 we see several of these basophilic masses of various dimensions; one of them begins to germinate and emits its first mycelial filament.

On the third day, many germinative masses have emitted long, highly ramified mycelial filaments (Fig. 5, pl. 114); all the ramifications laterally emit very sinuous, short, thin branches, less stainable by the dahlia than the large filaments and bearing zymospores or larger, short-pedunculated spores whose bedicel is spirally convoluted. The sinuous mycelial filaments detach very easily from the mycelium; the mass of the liquid is charged with an innumerable quantity of these mycelial filaments and the zymospores they carry (Fig. 6). The zymospores therefore have two origins: at the beginning they are formed on short, sinuous mycelial filaments emanating from the germinating spores; later they are also born on the mycelium emitted by the large germinative masses;

the various granules contained in the liquid are: either zymospores of this origin, or larger spores born on the mycelial filaments, or else granules formed by the fragmentation of the bacillary elements.

The fine mycelial branches segment and form straight or sinuous bacillary elements, comma-shaped or S-shaped, slightly basophilic; the larger, straight mycelial filaments segment, forming large, straight, very basophilic bacilli (Fig. 9) (Examine with a magnifying glass). The bacilli, fine or large, segment in turn until they reach the state of spherical granules.

Let us point out here that the bacterial culture thus formed is a culture of diphtheria bacillus, barley being the original source of this pathogen.

On the fourth day, the germinative masses, whose mycelial development has increased, form small colonies, or gather in clusters constituting the flakes visible in the liquid.

Such a colony can still progress following the evolution indicated at the beginning of this work for bacterial cultures; it can form a veil and this gives rise to an aerial gonidial form. The diastase of barley easily gives rise to the *Penicilium* form of the organic mold of the plant.

Culture and development of pepsin (Pl. 115).

A fresh solution of pig pepsin contains zymospores, spores and germinative masses (Fig. 1, pl. 115).

When inoculated in broth, the formation of agglomeration masses (Fig. 2) and their germination (Fig. 3) are observed after 24 hours. These masses emit mycelial filaments forming zymospores (Fig. 4). On the third day, flakes are formed which are large mycelial colonies (Figs. 7, 8); on the mycelial filaments of these colonies are born a multitude of spiral filaments ending in a spore or tortuous filaments carrying a series of zymospores. After four to five days the culture can form a veil which gives rise, first to ball-shaped fruiting bodies, then, later, to the *Penicilium or Aspergillus* form of the organic mold of pork.

II. Nature of Toxins and Antitoxins and Nature of Filtering Viruses.

Toxins are considered to be soluble bodies produced by bacterial elements in their culture or in the animal organism.

The following study demonstrates:

1. That toxins are constituted, not by soluble bodies, but by the formed elements, the zymospores of the culture from which they come.

2. That these zymospores are constant elements in any mold culture and that they are the ones that fulfill the fermentative role.

3. That they are subject to the same evolutionary phenomena as the other elements of molds and that they can reproduce them, entirely, in the form of hyphomycetes.

4. That the injection of a toxin into an animal develops the pathogenic agent in its organism as surely as the entire culture

5. That the zymospores, the active elements of bacterial toxins, pass through the porcelain filters due to their Brownian movement.

6. That they are carried along by centrifugation when, beforehand, an albuminous or mineral precipitate has been added to them.

7. That the filtering viruses are made up, not of elements invisible at high magnification, but of shaped elements from 0 μ1 to 0 μ8, the zymospores of the culture of the pathogenic agent, the latter also manifesting its presence in the organism by the other, usual and larger, elements of molds: bacteria, germinal masses, and mycelial elements.

8. That the prepared sera, called antitoxins, do not contain any element that destroys the antigen and do not prevent its development in the body, but only modify it; that they do not contain any immunizing element resulting from a defense reaction of the body.

Let us examine each of these points in turn.

1. *Toxins are not made up of soluble bodies, but of shaped elements, the zymospores of the culture from which they come.*

This is easily demonstrated with diphtheria and tetanus toxins.

The toxin is first examined at high magnification; a little is placed on a glass slide, dried at 60 or 70°, then stained with dahlia. Thus, the zymospores of varying size, which are photographed in Figures 1 (tetanus toxin) and 3 (diphtheria toxin) of Plate 116, are clearly visible.

If the toxin, with a little dahlia solution added, is examined between the slide and the coverslip, the zymospores are distinguished, exhibiting a lively Brownian movement.

Are these granules really zymospores, that is, the fermentative elements of the toxin or of the culture from which it is derived? If we examine in the same way a sample of the original broth in which the tetanus or diphtheria culture was inoculated, we see that it contains granules of the same size as the zymospores and also stainable by the dahlia; these are also zymospores; they were introduced into the broth by the peptone and by the beef.

Various means make it possible to demonstrate that the highest proportion of the zymospores of the toxin really belongs to the tetanizing element:

1. A drop of broth and a drop of toxin are spread respectively on two slides; after having spread them evenly over an equal surface, the zymospores from different fields of the preparation are dried, stained with dahlia, and counted or photographed. The toxin preparation contains a considerably larger number of zymospores than that of the original broth.

2. The test is much more demonstrative if the culture is inoculated into broth treated two or three times in succession with tricalcium phosphate and then centrifuged each time for 20 minutes at high speed. The broth thus obtained is sterilized in a centrifuge tube and centrifuged again the next day at high speed; only the upper two-thirds of the liquid is then decanted; the broth thus obtained still contains zymospores, but in consi-

derably fewer numbers than the original broth. The diphtheria culture is inoculated into it. The zymospores that appear there belong almost entirely to the culture.

3. A diphtheria culture inoculated into a solution containing 5/100 of glycose, *and completely deprived of zymospores or granulations,* develops there, but poorly; however, it develops sufficiently to form zymospores whose origin cannot be doubted.

Figures 10 and 11 of Plate 116 are photographs of a preparation of diphtheria culture in a 5% glycose solution; one will see, in the right part of Figure 11, the granules which are the zymospores constituting the toxin.

Other evidence is provided:

1. By the observation of the rarefaction of the zymospores after treatment of the toxin with tricalcium phosphate, coinciding with the reduction or loss of the properties of the liquid.

2. By the continuous examination of the toxin preserved at room temperature and in contact with air; the toxin progressively loses its qualities because the zymospores evolve, cultivate and reconstitute the original culture.

3. By careful examination, either of the evolving toxin or of the culture itself. *We see large spores or small germinative masses, formed by the fusion of several zymospores, emitting a fine, branched, tortuous mycelial filament, on which new granules identical to the zymospores develop.*

These various proofs therefore rigorously establish the identity and nature of the granules, animated by a lively Brownian movement, which the toxin contains and which constitute its active element.

2. *The figurative elements that are the active agents of the toxin are constant elements in any bacterial culture or in any mold culture; they are the ones that fulfill the fermentative role in the culture.*

These facts were demonstrated in a previous study on ferments; the diastase of germinated barley is constituted by the zymospores of the organic mold of barley. The diastase solution being made in sterile broth, preserved in the air and at room temperature, the zymospores it contains evolve, cultivate, and develop the bacterial culture characteristic of the organic mold of barley, then, later, an aerial mold that is a gonidian form of it.

* * *

3. The zymospores, the active elements of toxins, are subject to the same evolutionary phenomena as the other elements of molds: they can reproduce them completely, even in the perfect hyphomycete form.

This fact has already been demonstrated for certain ferments or enzymes, diastases from germinated barley, pig pepsin.

I have also transformed other ferments, notably trypsin, lab ferment, etc., into hyphomycetes. The same demonstration can be made with diphtheria and tetanus toxins preserved in the light and at room temperature; they transform and evolve spontaneously, after one to three weeks or less.

It is also possible, but less reliably, to develop the toxin on sugar agar (5% glycose, 5% maltose) by placing a drop or two of toxin on its surface at a single point, which is renewed several times and at the same point to accumulate the zymospores. The culture is kept at room temperature and in the light.

A culture of diphtheria and tetanus molds can also be obtained *quickly and reliably* by injecting the toxin into the guinea pig and culturing the animal's organs (lymph nodes of the injected leg, lung, liver, spleen).

Figures 4, 5, 6, 7, 8, and 9 of Plate 116 show the spontaneous development of diphtheria toxin in the three days following its manufacture, that is, the filtration of the broth culture through a candle.

The toxin is examined after filtration; Figure 3, plate 116, is a photograph of the zymospores that constitute it; their size varies from 0 μ3 to 0 μ6; the largest number is 0 μ5. There are other granules of larger size reaching 1 μ5 to 2 μm; these result from the fusion of several zymospores and are already germinative masses. This fusion constitutes the first phenomenon in the development of the toxin; it occurs within the first two or three hours (Fig. 4, plate 116) after filtration; the number of granules of 1 μ and above increases progressively, as does their size.

When the toxin is stored at room temperature and exposed to light, it is seen that, although very clear at first, it gradually becomes cloudy, and this cloudiness is greatest at the surface of the liquid, that is, in contact with the air.

A new examination of the liquid after twenty-four hours shows that the 0 μ 8 to 2 μ granules have germinated and emitted a short mycelial filament laterally forming short -pedunculated zymospores (Fig. 5, pl. 116).

We also note the appearance of very basophilic spherical agglomeration or germinative masses, much larger than the previous ones (2 to 6 μ.) (Fig. 6); in addition, bacilli have appeared in small numbers in the liquid, which means that a certain number of germinative masses have emitted mycelial filaments which have segmented.

After forty-eight hours, and then on the third day, the number and size of the germinative masses has increased again in certain points; the large masses (2 to 6 μ) are numerous (Fig. 9); the masses of 4 to 6 in turn unite into larger masses which emit numerous mycelial filaments segmenting into diphtheria bacilli; in fact, at this time, the number of bacilli in the culture is considerable; we can say that the culture is already completely constituted.

Preparations by hot desiccation of a drop of culture allow for better coloring of the elements and for obtaining clearer photographs; but the heat alters the shape of the elements; to observe these in their intact form, it is advisable to examine a drop of the living culture strongly tinted by the dahlia; the color of the liquid soon lightens, the dye gradually fixing itself on the represented elements. In a living culture thus prepared, we notice, in addition to the elements mentioned above:

1. Characteristic diphtheria bacilli.

2. Long mycelial filaments, not segmented, but partitioned and made up of articles placed end to end, each having the shape and structure of a diphtheria bacillus that it will constitute once detached (Fig. 7, pl. 116).

3. Germinative masses emitting mycelial filaments (Fig. 8, plate 116); the difficulty of observing such masses, with their mycelial filaments intact and still adherent, proves that these are very fragile and disintegrate under the influence of the slightest preparation maneuvers.

4. Large agglomeration masses of irregular shape consisting of both basophilic and hyaline elements. Such masses contain elements of all shapes and sizes (zymospores, bacilli, agglomeration masses), hyaline or basophilic.

In the same plate (plate 116) is contained (Fig. 1) the photograph of the figurative elements constituting the tetanus toxin.

It is known that tetanus toxin *deteriorates* and loses its properties if it is not stored in full, sealed tubes and away from light. This is how the toxin deteriorates when kept in contact with air, light, and room temperature. After two to three weeks, sometimes earlier (even on the second or third day), it becomes cloudy, especially on the surface,

when exposed to air. If the cloudy surface part is examined, it is seen to be made up of very large granules; some are grouped together to form germinative masses. If a new broth is inoculated with a drop of the cloudy liquid, it is found, after eight days (the culture being kept at 22°), that the culture gives off the nauseating odor characteristic of tetanus cultures and that it is in the process of bacterial proliferation. It contains mycelial filaments and bacilli of a new vegetative form of tetanus (Fig. 2, pl. 116). Injected in this state, the culture does not give tetanus to the guinea pig. The candle-filtered culture (i.e., the toxin of this culture) also does not possess the tetanizing power. The culture has therefore lost one of its pathogenic properties although it has retained some of its fermentative properties, a fact attested by the characteristic odor of the cultures **(1)**.

The toxin, thus spontaneously transformed into a bacterial culture of a new form by the evolution of its zymospores, makes it possible to obtain, with certainty and by successive transformations, the gonidian forms of the tetanus mold.

4. The injection of a toxin into an animal develops the pathogenic agent in its organism as surely as the total culture.

Diphtheria or tetanus toxin being injected into a guinea pig, the spleen, liver, and lung of the dead animal are reduced to a pulp and this is cultured on agar; after three or four days, the gonidial forms of the diphtheria or tetanus molds appear.

Another direct demonstration is given by the fact that anti-diphtheria serum makes it possible to reconstitute the diphtheria agent.

For this, we take serum that is two to six months old and has left a white deposit at the bottom of the bottle; we decant the serum completely to keep only the precipitate; we

collect this with a fine spatula and spread it on sugar agar, the culture being kept at room temperature; the characteristic diphtheria mold (*Citromyces* or *Penicilium*) generally appears after a few days.

5. Bacterial toxins do not lose the elements of their activity, the zymospores, by filtration on porcelain, because these have the property of passing through filters due to their Brownian motion.

This fact is easily demonstrated using ferments such as germinated barley diastase, lab ferment, pepsin, etc. The ferment is ground into powder in a mortar with a little 7.5%

(1) *The tetanizing power is, however, sometimes partially preserved.*

salt water to dissociate the zymospore clusters, then suspended in a sufficient quantity of liquid to obtain an opalescent emulsion.

The fermentative action of the solution is checked, on milk, for example, if working with lab ferment. It is filtered through very fine porcelain, then the fermentative power of the liquid is tested again; it is found to be preserved; *microscopic examination shows that the zymospores are present in large numbers in the filtered liquid.*

6. The zymospores, the active elements of ferments and toxins, are carried along by the precipitates due to a simple mechanical action and not by an adsorption phenomenon.

Ferments and toxins have been considered until now as soluble colloidal substances. They are in reality substances of a very different nature; they are spherical shaped elements, with an average size of 0 μm3 to 0 μm6 in general, formed by the organic mold specific to animals and plants. These shaped elements must be described by the general term zymospores, because they are in reality spores and it is they that carry out the fermentative reactions; their form, origin, and general properties are identical in both kingdoms, because every living being, animal or plant, is constituted by an organized mold, and the essential element in the evolution and nutrition of all mold, even unorganized, is the zymospore.

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We know that if a fine precipitate or emulsion of an organic substance that, for example, brain matter is added to a liquid containing ferment, it carries away (by centrifugation) the active elements of the ferment or toxin.

This phenomenon has been called adsorption; there would be fixation or attachment of the soluble active substance to the surface of the granulations of the precipitate.

This is only a hypothetical explanation, this fixation having never been demonstrated.
In reality, the entrainment of the active substance results from a purely mechanical action; the granulations of the absorbent precipitate are finer than the granulations of 0 μ 3 to 0 μ 6 which constitute the active element of the ferments and toxins. The multitude of granulations of the precipitate therefore constitutes a very fine mesh net which entrains the active granulations of the ferments and toxins when, by the effect of gravity (or centrifugal force), it falls to the bottom of the vessels. It is easy to see by stained microscopic preparations that, when a fine precipitate has been made to act in this way on a liquid rich in zymospores of a ferment or a toxin, the latter have decreased in number or disappeared in the supernatant liquid, and that they are present in the precipitate.

It is difficult to entrain all the zymospores from a liquid because of their lively Brownian motion; it is stated, regarding the spontaneous coagulation of fibrinogen solutions, that it is impossible to completely deprive them of the zymospores that constitute the serozyme.

7. Filtering viruses are constituted, not by elements invisible at the highest magnifications, but by the zymospores of the pathogenic agent, visible elements from 0 μ.3 to 0 μ.5.

A filtering virus is a virus which, after filtration through porcelain, retains its pathogenic properties; such a virus is said to be constituted by extremely small elements, invisible under the microscope. These viruses include those of foot-and-mouth disease, smallpox and vaccinia, measles, typhus, rabies, etc.

In reality, what has mainly led to the attribution of the ultra-microscopic constitution to filtering viruses is the fact that it has not yet been possible to isolate and cultivate the active agent of the diseases they cause; the active agents of these viruses having remained unknown and their pathogenic properties not being eliminated by candle filtration, it has been concluded that they are ultra-microscopic and invisible.

These notions are inaccurate; in fact:

1. The viruses reported so far as filtering, that is, as consisting of ultramicroscopic elements, do not differ in any way from other known viruses; all diseases with viruses reputed to be filtering are caused by a pathogenic mold that enters the body in the form of a bacterial culture. This fact is demonstrated for the main diseases with so-called filtering viruses, that is, foot-and-mouth disease, smallpox, vaccinia, measles, typhus, rabies, etc., in the last chapter of this work, which contains the description and representation of the molds that cause these diseases and their bacterial cultures.

2. The designation of filtering viruses cannot characterize the viruses considered so far as such, because, as a general rule, *all viruses are filtering.*

Every virus, whatever it is, appears in the form of a bacterial culture containing bacterial elements easily visible under the microscope (from 1 to 5 or 10 μ) and zymospores (from 0 μ to 3 to 1 μ).

The zymospores are capable, as well as the other elements, of transmitting the disease. When the culture is filtered through a candle, the large elements, averaging more than 1 μ, are stopped; the finer elements (zymospores) easily pass through the candle, thanks to their lively Brownian motion; the filtered liquid, which contains the zymospores, is what has been called a filtering virus for most diseases with an unknown

germ; it has been called a bacterial toxin when the filtered liquid is a culture broth of a known virus, the diphtheria or tetanus viruses for example.

For filtering viruses, we know that the fluid from a sick animal, filtered through a candle and inoculated into the animals, transmits the usual disease to them.

For known viruses, it has been believed until now that the filtered liquid, called toxin, exerts a different action on the animals than the culture. *This is an error;* it has just been demonstrated that diphtheria toxin and tetanus toxin, whose active elements are, like those of filtering viruses, *zymospores*, cause, when injected into animals, *not simple poisoning,* but diphtheria and tetanus; zymospores reconstitute in these animals the bacterial culture and the original pathogenic mold.

There is therefore identity between a toxin and a filtering virus; tetanus toxin is the filtering virus of tetanus; tuberculin is the filtering virus of tuberculosis. This means that the term filtering virus cannot be given to certain pathogenic agents and not to others; all the formed elements of a culture, whatever their size, can transmit the disease and reconstitute the entire virus in the animal organism; for all viruses, there exists a bacterial culture of uniform general constitution whose filtering part contains only the zymospores capable of reproducing the complete culture.

3. The active elements of the viruses designated until now as filtering (candle-filtered part of these viruses) are the zymospores; these are not ultra-microscopic or invisible elements, but elements of 0 μ3 to 0 μ6 or even up to 1 μ, stainable by basic colors and easily visible under the microscope; these zymospores exist in innumerable quantities in the cultures of the foot-and-mouth disease agent and in the candle filtrate of these cultures.

Composition of Antitoxins.

The name antitoxin refers to the serum of an animal that has received a series of injections of a toxin, that is, the filtered broth of a bacterial culture.

This designation of antitoxin was given to such a serum because it has been found that mixing it with the toxin considerably reduces its toxic power; the dose of tetanus toxin that kills a guinea pig in twenty-four hours becomes harmless, it is said, when mixed with a small quantity of antitoxin. In fact, the animal in this case shows none of the symptoms of tetanus and appears to have remained normal five or six days later.

It is believed that the power of the antitoxin results from the presence, in the serum of the prepared animal, of immunizing or protective antibodies created by a defense reaction against the injected toxin.

In the mixture of toxin and antitoxin, these antibodies would have the effect of neutralizing the toxin. Injected into an infected organism, the antitoxin would have the effect of destroying the bacterial elements of the pathogen.

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It would be impossible to establish the mechanism of these actions without first knowing the nature and composition of a toxin and an antitoxin; the nature of a toxin is now known: it is the zymospore of the pathogenic agent, endowed with fermentative power.

Let us now determine what the antitoxin is. I did this study on antidiphtheria and antitetanus serums.

It is known that these serums quickly deposit a white precipitate at the bottom of the flasks containing them; if the supernatant serum is carefully decanted and the precipitate is spread on sugar agar or cultured in broth, cultures are obtained that give rise to either the *Penicilium* form or other gonidial forms of diphtheria or tetanus molds; this same precipitate is inoculated into the guinea pig in 12 to 20 days; the pulp of the organs (liver, lung, spleen) of the animal is cultured on agar, and gonidial forms of diphtheria or tetanus molds are obtained in the cultures.

Anti-diphtheria and anti-tetanus serums therefore contain the formed elements capable of directly reproducing the pathogenic agents of diphtheria and tetanus. When inoculated into the guinea pig, these serums therefore give them tetanus and diphtheria.

This result was to be expected, because it has been previously demonstrated that the toxin, injected into an animal, does not act by determining only toxic effects; it acts by provoking the evolution of the disease itself by means of its zymospores which reproduce the pathogenic agent in its entirety.

Thus, when a horse is given a series of toxin injections, it is the very disease of the pathogenic agent that evolves in it, and it is from this evolution that immunization results. This fact was demonstrated above by reproducing tetanus and diphtheria molds by culturing on agar the pulp of organs of guinea pigs inoculated with the toxins of these pathogenic agents.

There can therefore remain no doubt about the following facts:

1. When an attempt is made to immunize a horse against diphtheria or tetanus by injecting toxin, it is given diphtheria and tetanus.

2. The horse serum thus prepared and delivered for medical use contains the figurative elements capable of reproducing diphtheria and tetanus when injected into the animal.

Diphtheria or tetanus contracted in this way do not develop like the same disease in the patient who contracted it through usual contamination.

The animals do not exhibit a lively acute reaction after the injection; for tetanus, they do not exhibit the muscular contraction and characteristic tetanic movements.

Since, obviously, the pathogenic mold develops in the animal injected with the antitoxic serum, it therefore appears:

1. That the development of the infecting mold does not occur in the same way, or with the same speed, when it is inoculated with the serum of a prepared animal.

2. That this difference must result from the fact: either that the prepared serum contains, in addition to the formed elements of the pathogenic mold indicated so far, another element which influences the evolution; or that these formed elements exist in the serum in a form or in a state different from the state which they naturally affect when they determine the usual disease.

New knowledge, resulting from other studies, will provide us with the means to determine, later, what is the cause of the special action of the serum of the immunized animal.

III. Study of Blood Coagulation.

In this study, we will examine in turn:

1. The physicochemical mechanism of fibrin formation in the blood, outside the vessels.

2. The mechanism of spontaneous coagulation of fibrinogen solutions considered pure.

3. The physiological significance of the phenomenon of blood coagulation outside the vessels; the physiological mechanism and purpose of the continuous coagulation of fibrin in the animal organism.

1. Physicochemical mechanism of the transformation of fibrinogen into fibrin.

It is generally accepted that blood coagulation results from the transformation of fibrinogen into fibrin by a ferment, thrombin or fibrin ferment.

Does the action of the fibrin ferment occur directly on the fibrinogen?

The study of the chemical phenomena occurring in the blood as it leaves the vessels shows:

1. That fibrin results from the fixation, on the fibrinogen, of a body with an acid reaction to phthalein.

2. That the fibrin ferment does not act directly on the fibrinogen, but is the agent that transforms the fatty substances in the blood (or the substances bound to these substances); it releases the substance which, by fixing itself on the fibrinogen, causes it to pass into the state of fibrin. Fibrinogen therefore plays only a passive role in the reaction.

The facts or observations that led me to these conclusions relate to:

1. To the study of changes in the reaction of the blood (acidity to phthalein) after it leaves the vessels.

2. To the determination of the nature of the body which, by its fixation on fibrinogen, forms fibrin.

3. To the study of the action of soaps and fatty acids on fibrinogen and on blood coagulation.

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Study of the reaction of blood after leaving the vessels.

The study of whole blood, from this point of view, is almost impossible. On the other hand, the study of the reaction of blood plasma, oxalated *as it leaves the vessels,* is very simple. The blood is centrifuged moderately to obtain a clear plasma, and two or three drops of alcoholic phthalein solution are added to it; the acidity is titrated using a sodium hydroxide solution.

The acidity of phthalein plasma is due to fatty acids or to a body bound to one or more fatty acids; I formulate this fact thus because agitation of the plasma with ether, which dissolves this body or the fatty acids, completely eliminates the acidity of phthalein. According to Doyon and Morel, the blood of the dog contains, per liter, 3 to 5 grams of organic acids combined in the state of ethers, approximately 0.50 grams of fatty acids combined in the state of soaps, and 0.32 grams of free organic acids.

A large portion of the organic acids combined in the state of ethers is found in the state of cholesterin oleate and palmitate (probably also stearate), that is, cholesterin soaps, the latter acting as a base. Another portion of fatty acids is combined with glycerophosphoric acid in bodies of the lecithin type.

The free organic acids are, at least in part, the fatty acids (oleic and palmitic) released by the phenomenon of dissociation in very dilute soap solutions; this phenomenon can be checked on a solution of sodium oleate at 0.5 g or 1 per 1000; we will see, at the same time, the increase in acidity to phthalein when the dissociation increases by the addition of an increasing proportion of salt, sodium chloride, for example. The addition of increasing proportions of sodium chloride to oxalated plasma or to serum determines the same phenomenon.

By acidity titrations carried out, either in normal oxalated plasma, or in oxalated plasma from the moment of recalcification, we observe:

1. That, in oxalated plasma, the acidity to phthalicin varies very slowly and decreases progressively over 24 or 48 hours.

2. That, in recalcified oxalated plasma, the proportion of free acid decreases rapidly and progressively until coagulation begins. Whether coagulation is slow or rapid, the maximum decrease in acidity always coincides exactly with the maximum coagulation.

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3. That from the moment the plasma is coagulated, *there is always an increase in acidity.*

As an example, here is the protocol of an experiment carried out using oxalated and highly centrifuged dog blood plasma.

Five cubic centimeters of oxalated plasma are poured into about twenty flasks. Four of these flasks are set aside as controls to measure their phthalic acidity. To each of the others, 3.5 cc of calcium chloride solution are added, a quantity very slightly greater than that sufficient to completely precipitate the sodium oxalate.

The phthalic acidity of a sample of unrecalcified oxalated plasma is measured at the moment of recalcification of the others; this same operation is repeated every thirty minutes on the other control samples.

The acidity of the recalcified samples is measured at variable intervals; for some samples, the assay is done in duplicate at the same time.

When coagulation has begun, it is necessary to break up the clot before the assay to completely remove the liquid, and to finely crush the pieces with a glass stirrer.

The table below contains the results of the assays, the acidity being measured by the quantity of sodium hydroxide solution (in teaspoons) at 1 in 1000 required for exact neutralization.

Time	Observations	Acidity of recalcified Oxalated plasma in CC NaOH 1p. 1000	Acidity of non-calcified Control oxalated plasma
0	Recalcification	0.90	1.20
3’30’	. . . . .	0.90	
7’	. . . . .	0.80	
11’	. . . . .	0.75	
30	Start of viscosity change.	. . . . .	1.20
60	. . . Total coagulation	0.40	1.10
70	. . . . .	0.60	
80	. . . . .	0.65	
90	. . . . .	0.60	1.25

Here, in a second experiment, is the value of the variations in acidity:

0	. . . . .	1.10	1.65
6’	. . . . .	1.10	
19’	. . .Total coagulation	0.55	
29’	. . . . .	1.00.	1.60

Let us also note in passing another result apparent in these two tables: when the oxalated plasma is recalcified, that is to say when the excess sodium oxalate is completely and exactly precipitated, the acidity of the plasma to phthalein undergoes an instantaneous decrease. The table below contains different measurements of this decrease carried out on different samples in 5 cubic centimeters of plasma.

Acidity of the oxalated plasma	Acidity immediately after recalcification
1.65	1.10
1.20	0.90
1.40	1.15
1.35	1.00
1.30 to 1.40	1.00

Thus, the acidity of phthalein-oxalated plasma, which corresponds on average to 28 (the number of cubic centimeters of 1% sodium hydroxide solution needed to neutralize 100 cc of oxalated plasma), instantly falls to 21 when the action of the oxalate is eliminated by precipitating it.

If we methodically extract from these facts the indications they provide, we see:

A. That in the period preceding coagulation, the fall in the acidity curve indicates that there is fixation, on a substance, of an acid body existing in the plasma.

B. That from the moment coagulation occurs, *that is to say, when fibrin is formed,* the cessation of the fall in the acidity curve, and even more so its rise, signify that the substance that fixed the acid body no longer fixes it or fixes it much less.

There is therefore a direct link between the formation of fibrin and the fixation of the acid body, the fibrin resulting from this fixation on something which, we will see later, is fibrinogen.

C. That the increase in acidity which follows coagulation proves that at this moment there is, in the blood, *a continuation of a phenomenon of formation or release of a body with an acid reaction to phthalein.* This release having been at least as active from the exit of the blood from the vessels until the moment of coagulation as after it, we can therefore conclude:

That the free quantity of this body, which was low in the blood contained in the vessels, since it gradually fixes itself to the fibrinogen, has increased notably by this new formation. This body having fixed itself for the most part to the fibrin, it is therefore from this substance that it must be extracted. As it continues to form after coagulation, some therefore remains in the serum. This is in perfect agreement with known facts. The blood does not contain thrombin when it leaves the vessels. It does contain some after a certain time; the clot is rich in thrombin and the serum still contains a certain quantity.

D. That, in oxalated plasma, the fixation and release of the acid body are prevented or considerably reduced by the presence of oxalate.

If oxalated plasma manages to coagulate in the long run after one or two days or more, it is because the normal mechanism of the coagulation phenomenon operates despite the presence of oxalate, but with a considerable slowdown.

E. That the phenomenon of fixation of an acid body in coagulation is indeed linked to the formation of fibrin, since the prevention of coagulation determined by oxalate coincides with a prevention of the fixation of the acid body. Since the fixation of the acid body is indeed linked to the formation of fibrin, it is therefore because this fixation occurs on fibrinogen.

Determination of the nature of the acid body which, by its fixation on fibrinogen, forms fibrin.

The preceding determinations demonstrate that there is formation in the blood, as soon as it leaves the vessels, of a body with an acid reaction to phthalein which accumulates on the substance which will constitute fibrin, that is to say on fibrinogen.

The real existence of this body and its acid function can be demonstrated by the following methods:

1. If oxalated plasma is violently agitated with an equal volume of ether for ten minutes each time and four or five times in succession, the acidity to phthalein disappears completely or almost completely. The plasma thus treated no longer coagulates upon the addition of calcium chloride, or its coagulation is very slightly accentuated and slowed down. The ether collected and evaporated leaves behind a body which, agitated with water, precipitates a solution of fibrinogen.

2. Five cubic centimeters of recalcified oxalated plasma are introduced into five flasks. One is kept as a control. To the other four, 0 cc, 2-0 cc, 4-0 cc, and 5-0 cc, 6 of 1 part sodium hydroxide solution are added, respectively, and the coagulation rates are observed. The results are recorded in the table below:

Nos	Quantity of 1% NaOH added	Observations
1 (control)	0	Complete coagulation in 20 min.
2	0.2	Not coagulated after 1.5h; soft clot after 2h.
3	0.4	Beginning of coagulation after about 6h. Soft clot after 7h; hard clot after 7.5h.
4	0.5	No traces of coagulation after 10h; found coagulated after 20h.
5	0.6	No traces of coagulation after 10h; soft clot after 20h.

This experiment proves:

A. That the addition of a base does not prevent the release of the acidic substance from continuing.

B. That it delays coagulation, especially as the quantity added increases.

C. That the added base fixes the acidic substance formed and prevents it from transferring to the fibrinogen to transform it into fibrin.

D. That the reaction that transforms fibrinogen into fibrin (fixation of the released substance to the fibrinogen) is independent of the reaction that releases the transforming substance.

Fibrinogen plays only a passive role. It remains unchanged as long as another substance (soda) fixes the released body; when this substance is saturated, it in turn fixes the released body until coagulation. The proof of this fact is contained in the experiment; to verify it, it is sufficient to add as an indicator to each of the flasks nos. 2, 3, 4, and 5, one or two drops of alcoholic phthalicin solution, which gives the plasma a strong pink tint. When this pink tint disappears, that is, when the soda is saturated, coagulation begins shortly after.

3. By prolonged dialysis of the oxalated plasma for 15 or 20 hours, the acidity to phthalein disappears notably. This serum, resalted in the proportion of 7.5 p. 1000, no longer coagulates, neither spontaneously nor by the addition of calcium chloride, while the control oxalated plasma coagulates if the oxalate is completely precipitated by CaCl2. The acid body has disappeared either by dialysis or by combination with certain constituents of the plasma.

Dry fibrin being exhausted by alcohol or ether, these leave behind by evaporation a body which, agitated with water, instantly precipitates a solution of fibrinogen in very dense fibrinous lumps. The precipitating body thus isolated from fibrin by ether appears to owe its properties to glycerophosphoric acid and to be one of the fragments of hydrolyzed lecithins.

In possession of these indications, I did not seek to know immediately the chemical constitution of the body thus extracted from the fibrin or from a clot by alcohol, because, in the first place, this solvent removes several bodies from the clot and not just one; in the second place, because the determinism of the formation of fibrin was not sufficient.

Action of soaps on blood clotting.

To complete this determinism, I investigated whether blood soaps come into play in the phenomenon of fibrin coagulation because, added in the right proportion, they accelerate coagulation.

Indeed:

1. If increasing quantities of a saturated solution of neutral sodium oleate (8 to 10 p. 100):

A. Coagulation is greatly accelerated at an optimum proportion of 0.60 grams of sodium oleate per liter of blood; complete coagulation occurs in two to five times less time than in normal blood.

B. Beyond this optimum proportion, the accelerating effect decreases. When the proportion of 3.5 to 4 grams per liter is reached, the blood becomes uncoagulable.

C. The accelerating effect of sodium oleate is cancelled if the oleic acid is released by the addition of an acid. Such an oleic acid emulsion also does not exert the same anticoagulant effect; this effect becomes barely noticeable.

2. The dropwise addition of a saturated solution of sodium oleate to a solution of fibrinogen appears to precipitate it into fine lumps or flakes which settle; the sodium oleate solution, transformed into a fine emulsion of oleic acid by an acid, loses its precipitating power.

I sought to determine the mechanism of this action by studying it on fibrinogen solutions. This study did not provide any information that significantly clarifies the question. I found that the precipitate that forms by gradually adding, or all at once, a saturated solution of sodium oleate to a fibrinogen solution, consists of soap that has become insoluble, that is, acidic, and not of the fibrinogen; the soap therefore seizes part of the acid body fixed on the fibrinogen. This fact does not shed any light on the mechanism of the clearly favorable action of sodium oleate on blood coagulation. It is therefore only to the action of the released acid body that we must attribute the coagulation of fibrinogen.

The origin of this acidic body appears quite clearly. We know that there is a normal phenomenon of lecithin hydrolysis in the blood; this phenomenon releases fatty acids and glycerophosphoric acid and continues in the serum after the formation of the clot; indeed, analyses of the blood after it leaves the vessels have shown (**Doyon** and **Morel**) that the quantity of soaps and organic acids (mainly fatty acids and glycerophosphoric acid) increases progressively, while a progressive and correlative decrease in lecithins occurs.

* * *

The sequence of phenomena that we have determined and of phenomena already known is therefore as follows:

1. There exists in the circulating blood, and also in the blood leaving the vessels, a normal, continuous phenomenon of lecithin hydrolysis. This phenomenon releases fatty acids and glycerophosphoric acid.

2. A body with an acid reaction to phthalein is released into the blood continuously, immediately after it leaves the vessels.

3. This acidic body to phthalein is fixed, as it is formed, on the substance which, by coagulation, will constitute fibrin. As soon as the fixing body, fibrinogen, is coagulated and passes into the state of fibrin, the proportion of the acidic body in the blood which, until then, had gradually decreased, varies in the opposite direction, that is to say, increases progressively because the absorption capacity of the fixing body is largely satisfied.

It, thus, becomes very apparent:

1. That the immediate cause of the transformation of fibrinogen into fibrin is the fixation of the acidic body to phthalein.

2. That the acidic body formed is probably glycerophosphoric acid or the portion of hydrolyzed lecithin which comprises the acid group.

The accuracy of these deductions is confirmed by the coagulating action exerted by lecithin added to blood or plasma. Glycerophosphoric acid is a strong coagulant of fibrinogen; it is true that when added to plasma, it does not coagulate it. But this fact does not invalidate the preceding conclusions, because fibrinogen is present in plasma with other colloids that modify its reactions, and because the artificial addition of glycerophosphoric acid to plasma in no way achieves the conditions for continuous separation, in the nascent state, of the acid portion of lecithins in their hydrolysis.

2. Mechanism of spontaneous coagulation of fibrinogen solutions reputed to be pure.

According to currently accepted notions, blood coagulation results from the action of a ferment, thrombin or fibrin ferment, on fibrinogen.

This thrombin itself is made up of two bodies: one, cytozyme, existing in the blood and in all tissues, is, according to Bordet, a lecithin; the other, serozyme (Bordet), is a blood ferment found in serum.

Cytozyme would be formed in the blood by platelets; but a simple lecithin solution could replace it and exert the same action.

It is easily perceived that what has been called thrombin are the elements of the hydrolysis phenomenon, that is to say, the hydrolyzing agent (serozyme) and the hydrolyzed body (cytozyme).

Until now, these two elements have been considered linked, even in the complex state. It was not thought that, one being a lecithin, the other a ferment, they constituted the two indispensable elements for a hydrolysis phenomenon, and that by reacting together they could give rise to new bodies intervening to determine the coagulation of the blood.

It is clear from the preceding determinations and explanations that thrombin is not the group of two factors in the hydrolysis phenomenon (serozyme, cytozyme), but one of the products of the hydrolysis of cytozyme: the acid portion of lecithin.

The existence of serozyme is not established by the isolation of this body; its existence is admitted by deduction drawn from the enrichment of serum with thrombin when lecithin is added to it. The enrichment of serum with thrombin by the addition of lecithin means that the coagulating power increases by the formation of a new quantity of the hydrolysis product possessing the coagulating action.

It is admitted that thrombin cannot be formed, in the absence of lime salts, in a medium decalcified by the addition of sodium oxalate.

Current coagulation theory has difficulty explaining a known fact: if fibrinogen is prepared as pure as possible **(1)**, fibrinous flakes form after a certain time. Bordet attributes the formation of these flakes to the presence of serozyme; he succeeded in delaying their formation in a solution of fibrinogen deprived of serozyme by adding tricalcium phosphate followed by centrifugation. But this process only delays the formation of the flakes; it does not prevent it. The phenomenon is not limited to the formation of these flakes: *the fibrinogen eventually coagulates spontaneously,* en masse, forming the typical fibrin clot.

Thrombin has therefore formed in the solution.

Since fibrinogen was prepared in a decalcified medium and has not been recalcified, its coagulation therefore establishes, first and foremost, without any reservation, that lime is not necessary for the formation of thrombin.

On the other hand, cytozyme (lecithin, which is said to be contained in blood platelets) and serozyme appear to have been almost completely eliminated by the addition of tricalcium phosphate; *coagulation occurs even if this eliminative treatment has been carried out three times in a row on fibrinogen*; to see the latter coagulate spontaneously and fairly quickly, it is sufficient to dissolve it in as concentrated a solution as possible and with the minimum necessary salt.

(1) *It is prepared as indicated by Bordet, using vigorously centrifuged oxalated plasma and purifying it by several successive precipitations preceded each time by treatment with tricalcium phosphate.*

In the presence of such a result, blood coagulation becomes inexplicable by the action of thrombin, that is, of the serozyme, cytozyme group.

Indeed, Bordet found that the addition of serozyme alone does not accelerate the coagulation of pure fibrinogen solution, while the addition of platelets alone (which is considered to be the cytozyme of blood) clearly activates it, even though it should have no effect.

It seemed to me very likely that the determinism of this phenomenon should lead to knowledge of the mechanism of blood coagulation.

This determinism has indeed led to an incomplete, but already sufficient, knowledge of the mechanism of coagulation and, in addition, of its physiological significance. It was achieved through the following successive research:

1. Study of the normal formed elements of whole blood, then of those which remain in the oxalated plasma after a first centrifugation, then after a second, more vigorous centrifugation; this study was repeated for the serum.

2. Determination of the effect of the addition of tricalcium phosphate to plasma on the elements it contains; repetition of this study on serum.

3. Microscopic study of a fresh fibrinogen solution and after several successive centrifugations.

4. Microscopic study of the flakes that appear spontaneously in a fibrinogen solution.

5. Microscopic study of the clot formed by the spontaneous coagulation of fibrinogen.

6. Microscopic study of the clot of spontaneously coagulated blood, immediately after leaving the vessels.

Here are the observations made on each of these points:

1. Study of the normal formed elements of whole blood.

This study has already been explained on page 147. I will not return to it here. I will simply indicate that all the elements of the blood are involved in coagulation, but that those that play an active role are:

A. Hematoblasts

B. Granulations or spores of various sizes, mainly zymospores from 0 µ3 to 0 µ5. These various elements are photographed in plate 108.

When oxalated blood is centrifuged moderately, most of the large elements fall to the bottom of the vessel (red blood cells, white blood cells, hematoblasts, branched mycelial filaments loaded with zymospores, a certain quantity of granules of various sizes).

In the oxalated plasma, hematoblasts, a certain quantity of mycelial filaments loaded with zymospores, and granules remain. In a new, very vigorous centrifugation, this plasma abandons a new portion of all these elements, notably the hematoblasts, almost all of which are deposited.

But there is one element from which centrifugation does not remove the plasma. These are the 0.3 to 0.5 μm zymospores, which remain suspended in the liquid thanks to their Brownian motion.

In the serum, we find the same elements that have escaped from the fibrin framework. Vigorous centrifugation separates them quite easily from the serum, with the exception of zymospores of 0 μ3 to 0 μ5, which remain in suspension due to their Brownian motion.

2. *Effect of the addition of tricalcium phosphate to plasma or serum.*

These are liquids that have already been heavily centrifuged. The gelatinous precipitate of tricalcium phosphate, added to these liquids, traps a large number of zymospores and drags them to the bottom of the tube by centrifugation. They are found in the tricalcium phosphate pellet, and it is noted that, in the liquid, their number has greatly diminished. But a single addition does not completely deprive it of them; some still remain, even after two successive operations.

The action of the tricalcium phosphate precipitate is therefore not due to an adsorption phenomenon, but simply a mechanical phenomenon.

The granules of the precipitate trap the zymospores, which are larger than themselves, and drag them by their weight to the bottom of the tube. Due to their Brownian motion , many zymospores escape from the precipitate and remain in the supernatant liquid. The action of the precipitates on the ferments and toxins is identical and of the same nature, these being also constituted, not by soluble substances or invisible colloidal granules, but by zymospores of 0.3 to 0.5 μm and even by other larger granules. Since serum contains far fewer zymospores than plasma, it is easier to deprive it of them by adding tricalcium phosphate, but a single addition of this substance, or even two successive ones, does not remove them all; a small number always remain.

3. Microscopic study of a fibrinogen solution.

Let us prepare fibrinogen with vigorously centrifuged oxalated plasma precipitated by adding 21 grams per 100 of NaCl (or 17 grams per 100 if desired). Let us precipitate it twice more after redissolving it, and wash the precipitate each time with salt water. Redissolved again and examined under high magnification, we see that it contains zymospores of 0.3 to 0.45 μm and even larger granules. A portion of the solution, set aside, contains fibrinous flakes the next day and coagulates spontaneously in one to three days.

If we centrifuge the remaining solution after adding tricalcium phosphate, we see by microscopic examination that the number of zymospores has greatly decreased. A portion of this solution forms lumps much more slowly and only coagulates after four to six days, for example.

A further addition of tricalcium phosphate further rarefies the zymospores and delays the moment of spontaneous coagulation, but does not prevent it. I obtained coagulation of fibrinogen solutions, even after a third operation of this kind, but I found that it occurred without clear flakes forming in the solutions. We will have the explanation of this fact later.

It was Bordet and Delange **(1)** who first indicated the means of make fibrinogen solutions more stable by adding tricalcium phosphate. The effect of tricalcium phosphate is very clear, but, however, I observed a stabilization of less duration than that indicated by Bordet and Delange. These authors wondered what principle is absorbed by tricalcium phosphate to produce this effect; they do not believe that it is thrombin or its generators, cytozyme and proserozyme.

It is the zymospores (serozyme, proserozyme does not exist) whose entrainment gives the fibrinogen solution greater stability.

4. Microscopic study of fibrinous flakes that form in a fibrinogen solution.

The fact that a decalcified fibrinogen solution coagulates without the addition of lime proves that the latter does not intervene in coagulation. Such a solution, deprived of cytozyme and also deprived of serozyme, it is believed, by three successive additions of tricalcium phosphate, also deprived of lime, nevertheless coagulates spontaneously and without any intervention.

(1) *Bordet and Delange, Bull. Soc. Sc. Méd. et Nat. de Bruxelles, 1914.*

The determination of this phenomenon can be made, in part, by the microscopic examination of the flakes that form in fibrinogen solutions; I turned, to begin with, to a solution not treated with tricalcium phosphate.

Having spread on a slide, dried and then stained with dahlia, one of the first fibrinous flakes formed about forty-eight hours in the solution, I noted with surprise, upon examining it, that it was made up of a voluminous and very developed mycelial colony. Carefully examining the elements of this colony, I was able to observe that masses of fibrin were forming at certain points, and that they were constituted exactly like the coagulations and condensations of hyaline matter which constitute the beginning of the formation of germinative masses in bacterial cultures and in colonies of fungi.

These masses of fibrin were, for example, identical to fibrinous masses of variable volume, often considerable, which are formed in the entanglement of mycelial filaments or on the periphery of colonies of *Fusarium solani* for example; these masses are represented in figure 1, plate 117. At other points, a mass of long parallel filaments (Fig. 2), grouped by a fibrinous mass, extending far from the colony.

Looking in other points, I found the fruiting bodies of the mold, long elongated spores like *Fusarium gonidia* (Fig. 3). Finally, at high magnification, I observed, in the intervals of the mycelial filaments and in the fibrinous masses, a considerable quantity of spores and zymospores, of all the dimensions, encountered and described here previously in the blood.

Studying other fibrinous flakes of various sizes, included in fibrinogen already completely set in jelly, I found them all to be made up of analogous mycelial colonies or presenting their mycelium in the different aspects represented in Plate 118).

Some gelatinous masses were formed by large, sinuous and tangled mycelial filaments at certain points (Figs. 1, 2, 5, pl. 118) and, at others, grouped in parallel in long, compact bundles containing a considerable number of filaments (Figs. 2, 5, pl. 118). The fibrinous extensions of the colony were all inhabited by characteristic mycelial filaments (Fig. 6, pl. 118). At many points, clusters of bacilli or fruiting bodies formed by the mycelial filaments were observed (Fig. 7, pl. 118).

5. Microscopic study of the clot formed by the spontaneous coagulation of fibrinogen.

What caused me the most astonishment was certainly the appearance of the colonies vegetating for several days inside the clot of fibrinogen, coagulated en masse and prepared either without treatment with tricalcium phosphate or with the addition of precipitate only once. A rapid examination revealed at once that what I had before my

eyes, which had just formed with only a solution of fibrinogen and a small number of spores and zymospores from horse blood, was connective tissue; and it was inside and around the mycelial colonies that led to this connective tissue that the fibrin coagulated and was deposited.

It thus appeared that it is the organic mold of the animal, from which the fibrinogen originates, which thus develops into colonies forming the flakes, and that the coagulation of the fibrin around them is a phenomenon identical to the coagulation of hyaloplasma in molds cultivated in vitro.

The formation of fibrin is therefore a general phenomenon having as its aim the development of the formed elements of the mold fissure, especially of its mycelium; when it is an organized mold constituting an animal, the formation of fibrin has the aim of the development of tissue elements or their repair.

There is no doubt that this is indeed connective tissue in the present case.

Plate 119 contains photographs of four different regions of the same colony. Under a magnifying glass, one will find the elements of this tissue, the bundles of connective fibers with their ramifications, their fruiting bodies, the beginning of the formation of fixed cells, the germinative masses, etc.

Figure 4 contains (in the center) a large germinative mass from which a considerable mass of parallel connective fibers originates, each of them being the germinative filament of one of the spores constituting the agglomeration. This figure explains to us how a large connective bundle can contain, as we see, such a large mass of parallel fibers and confirms the origin of these fibers already indicated; it explains to us the presence and origin of the enormous masses of parallel hyaline fibers (Fig. 4, pl. 118) constituting the fibrinous filaments emanating from the flakes.

Let us now study the origin of these and determine the elements which give rise to them.

It has been explained above that fibrinogen, even if it is treated two or three times in succession with tricalcium phosphate, always retains a certain quantity of spores and zymospores These form, by their agglomeration, germinative masses which emit mycelial filaments loaded with new zymospores, a fact demonstrated later. This is the explanation for the repopulation of the fibrinogen solution with zymospores. These in turn form germinative masses larger than the first and which are the origin of the first colonies which appear in the form of fibrinous flakes. The fibrin is first deposited at the level of these colonies, because they are the site of an intensive formation of new zymospores.

Coagulation then takes place throughout the mass of fibrinogen, because the zymospores distributed throughout the liquid have formed in sufficient number to cause coagulation.

The proof that this is indeed the case and that it is they that cause coagulation is that, to prevent a fibrinogen solution from coagulating, it is sufficient to treat it with tricalcium phosphate every two or three days, that is to say, to carry away the zymospores as they reform.

The mode of formation of zymospores is easy to observe in a fibrinogen clot. To do this, we use a solution of fibrinogen treated twice in succession with tricalcium phosphate. It is allowed to coagulate spontaneously, then a small fragment of the clot is crushed between two slides. It is allowed to dry in the cold, stained with dahlia in the cold and washed in cold water.

We can thus see that the clot is entirely formed by a felting of a multitude of small mycelial colonies (Fig. 1, 2, 3, 4, pl. 120) each consisting of a small germinative mass *identical to a blood hematoblast* and emitting sinuous mycelial filaments loaded with zymospores. The fibrin formed unites and adheres these colonies together, leaving free spaces filled with liquid. The liquid that flows by tearing or crushing the clot is that of these areolae or tearing free spaces.

What forms the fibrinogen clot is therefore an agglutinating substance that unites the developed mycelial elements together in a felting. This substance, called fibrin for blood, does not differ, in terms of its physiological role and mode of formation, from the agglutinating substance that forms in any bacterial or mycelial colony and which, once deposited on the surface of its elements, determines their agglomeration. This perfect identity recognizes as a cause the identity of the elements of the culture medium called blood, with those of an in vitro bacterial culture medium. In both cases, it is a mold that vegetates in the medium and develops there, through its zymospores, the hyaline substance (fibrin, hyaloplasma) necessary for its growth.

These facts establish the nature, origin, and mode of formation of the bodies called *agglutinins.* These are the zymospores and spores of a fungus; these formative elements must be distinguished from the substance formed by them, the agglutinating matter which, in the organism, is blood fibrin and which, in an in vitro culture, can be called bacterial fibrin or mycofibrin.

The agglutinating matter formed by the zymospores of a species of hyphomycete is deposited only on its elements and agglutinates only them.

We will see later that the phenomenon of precipitation is identical to that of the formation of flakes or mycelial colonies, and that the body called precipitin is made up of zymospores or other formed elements of the sensitizing heterogeneous body, developed in the blood and subsequently transferred to the sensitizing solution.

6. Microscopic study of spontaneously coagulated blood, immediately after leaving the vessels.

Coagulation occurs in the blood leaving the vessels, generally within two to 5 or 10 minutes. It is unlikely that the mycelial arborizations, whose intricacy constitutes the fibrinogen clot, have time to form in the blood leaving a vessel until the moment of its coagulation. Does the blood clot nevertheless have the same constitution as that of fibrinogen? This can be determined by microscopic examination of the fresh clot as follows: prick the ear of a guinea pig and place the drop of blood that appears on a glass slide. As soon as it is coagulated, the clot is dragged with a needle, moving it over the slide to make it release as much blood as possible.

A small fragment is detached, crushed between two glass slides, dried, and stained with dahlia. Figures 5 to 9 of Plate 120 represent photographs of preparations thus obtained.

These photographs show:

1. That the blood clot has the same constitution as a fibrinogen clot and is formed by the agglomeration of a considerable number of fine mycelial arborizations loaded with zymospores.

2. That these mycelial arborizations emanate from the hematoblasts that give rise to them, or from isolated spores emitting a single undulating mycelial filament carrying zymospores.

The mycelial arborizations formed either by isolated spores or by hematoblasts are visible in Figures 5, 6, 7, 8, 9, Plate 120; the barely colored and torn fibrin framework is visible in Figures 5, 7, 8, 9. Figure 5 shows a mass of blood cells, hematoblasts, and arborizations agglomerated by fibrin. At many points the mycelial arborizations formed by the hematoblasts are clearly visible, notably in Figure 9 where one of them is isolated.

Here, coagulation is immediate, because the number of zymospores contained in the blood is considerable and sufficient. The clot has the same constitution as a fibrinogen clot, because the mycelial arborizations are all formed. The preparations made as just indicated even constitute an excellent means of demonstrating their existence surely and quickly.

Let us now group together the information provided by the study of fibrinogen coagulation and the constitution of the fibrin clot; they can be summarized as follows:

1. The portion of thrombin called serozyme is made up of blood zymospores, elements of 0.2 to 0.5 microns driven by Brownian motion. *Because of their Brownian motion, the zymospores always remain suspended in the blood, plasma, or serum, and it is impossible to separate them from liquids by centrifugation.* They are partly carried along with the precipitates and resuspended when they are redissolved.

2. The blood zymospores are carried along with the fibrinogen precipitates. Treating fibrinogen solutions with tricalcium phosphate deprives them of a large portion of their zymospores, but is incapable of carrying them all away.

3. Zymospores are fermentative elements of the specific organic mold; in addition to fermentative power, they possess vegetative and reproductive power. Remaining in a fibrinogen solution, even in very small quantities, they undergo the evolution of all bacterial elements; they germinate directly or form small germinative masses that emit mycelial filaments on which new zymospores are born.

The serozyme, very rarefied in fibrinogen solutions by the action of tricalcium phosphate precipitates, is therefore reconstituted there by multiplication of the elements that escaped this action.

4. The vegetation of zymospores results, in addition to their multiplication, in the in vitro formation, in fibrinogen solutions, of agglomeration masses or germinative masses identical to the hematoblasts of the blood and to larger elements similar to blood cells.

5. When the multiplication of zymospores is sufficiently advanced, even larger germinative masses are formed, giving rise, in vitro, to mycelial elements identical to those of the organism's connective tissue.

These mycelial formations, isolated or in groups, as well as the clusters of germinating spores or zymospores, constitute the flakes that appear and grow in fibrinogen solutions.

6. In fibrinogen solutions, fibrin is always formed and deposited first on the elements of the mycelial colonies or flakes; these elements are included in a mass of fibrin that serves for their formation.

7. Blood platelets are made up of hematoblasts, agglomerated masses of spores and zymospores.

A platelet emulsion is a liquid containing, at the same time, hematoblasts, agglomerated masses of spores and zymospores, isolated spores and zymospores, and various bacterial elements belonging to organic mold.

Since these various elements are alive and capable of vegetation and multiplication, a platelet emulsion is therefore completely different from a lecithin solution obtained by dissolving it in alcohol, filtering it, evaporating it, and suspending it in water; it cannot in any way be called, like this solution, by the name of cytozyme, because it cannot be assimilated to it.

8. An aqueous juice or tissue emulsion, which exerts an action identical to a platelet emulsion, contains a considerable quantity of zymospores; it is these that give it its energetic coagulating power.

9. Platelets, like tissue zymospores, contain lecithin. These elements are therefore capable of acting in two different ways: either as agents for multiplying zymospores, that is, producing serozyme, or as a lecithin emulsion or cytozyme in solutions where the vegetative power of the multiplying elements is destroyed by boiling.

From these facts, we possess almost complete knowledge of the mechanism of spontaneous coagulation of fibrinogen solutions reputed to be pure.

The fibrinogen precipitate carries away zymospores that constitute serozyme, which tricalcium phosphate cannot carry away in their entirety. In the redissolved fibrinogen, these zymospores vegetate and reproduce serozyme, the proportion of which gradually increases, causing coagulation when it is sufficient; this explains why tricalcium phosphate delays coagulation.

But the presence of fibrinogen and serozyme in the solution is not enough to ex-plain coagulation, since we have previously established that fibrinogen passes into the state of fibrin because it fixes an acid body resulting from the hydrolysis of a lecithin (cytozyme) by a ferment, serozyme.

In the fibrinogen solution considered pure, the serozyme reforms by multiplication of the zymospores, but this hydrolyzing ferment would be powerless to form the acid body determining coagulation if the hydrolyzable body, lecithin, does not exist in the solution.

It must therefore be concluded that either the zymospores (serozyme) act on a lecithin carried by the fibrinogen precipitate, or that they are capable of transforming part of the fibrinogen to reconstitute lecithin or a similar body.

It is obvious that a transformation action exists since a notable quantity of vegetative elements is formed in the solution and this formation, which requires an albuminous substance, can only occur at the expense of fibrinogen.

It also seems likely that fibrinogen carries with it a certain quantity of lecithin; indeed, *lecithin is partially carried out of its solutions by the precipitates.*

A solution of fibrinogen considered pure therefore coagulates by a phenomenon identical to that which determines the coagulation of blood. Fibrinogen carries with it, in its preparation, lecithin and the elements (zymospores) capable of reforming a sufficient quantity of serozyme. If the coagulation of fibrinogen is slow and all the slower the more energetically its solution has been treated with tricalcium phosphate, it is because it takes time for the vegetation, or culture of zymospores, to reproduce the serozyme in sufficient quantity; this time is all the longer the smaller the quantity of zymospores remaining in the solution.

This explains why lecithin (cytozyme), added to a fibrinogen solution, does not accelerate its coagulation, while a platelet emulsion has this effect (Bordet) **(1)**. This is because the fibrinogen solution contains enough lecithin for its coagulation and because a platelet emulsion differs completely from a lecithin emulsion; it is an emulsion of vegetative elements. It brings into the solution the elements capable of rapidly developing zymospores, that is, serozyme.

3. Physiological significance of blood coagulation outside the vessels. Mechanism and purpose of the continuous coagulation of fibrin in the animal organism.

The demonstrations made so far provide us with knowledge of the mechanism of blood coagulation outside the vessels. This mechanism is as follows:

1. There exists in circulating blood, as in blood leaving the vessels, a continuous phenomenon of hydrolysis of lecithins, bodies designated until now by the word cytozyme.

2. The hydrolysis is carried out by a ferment called serozyme, consisting of the zymospores of the animal's organic mold contained in the blood.

(1) *BORDET, SOc. de Biologie, page 576, 1920.*

3. The hydrolyzing action of serozyme has the effect of releasing a body with an acid reaction to phthalein, which is probably the acid portion of hydrolyzed lecithins

4. The fixation of the acid-reacting body to phthalein on fibrinogen is the direct cause of its transformation into fibrin.

From these facts it follows that the fibrin ferment or thrombin, which has been considered as a complex of two bodies, the cytozyme and the serozyme, is neither the immediate coagulating agent nor a complex; the cytozyme (lecithin) and the serozyme (zymospores) are the two elements of the hydrolysis phenomenon; the direct coagulating body is one of the products of this phenomenon; it is therefore this which must bear the name thrombin, and not the two elements of the hydrolysis phenomenon, the lecithin (cytozyme) hydrolyzed body, and the serozyme (zymospores) hydrolyzing body.

The hydrolyzing action of zymospores on blood lecithins is a normal physiological phenomenon that occurs and continues slowly in the circulating blood and inevitably continues after it leaves the vessels.

If the coagulating acid body attaches itself to fibrinogen and coagulates it in the blood leaving the vessels, it is therefore also attaching itself to it and coagulating it in the circulating blood.

Thus it appears that the formation of fibrin or blood coagulation, outside the vessels, is not a phenomenon of alteration or cadaveric. It is a normal and continuous physiological phenomenon in the body.

The formation that we have observed of the elements of connective tissue in a solution of fibrinogen in vitro, and with *this substance passing into the state of fibrin, demonstrates that the coagulation of fibrinogen has as its goal the formation and renewal of the anatomical elements of the tissues,* that is to say, the formation of the vegetative elements of the organic mold.

In the blood leaving the vessels, coagulation occurs en masse *because the blood is no longer in the presence of the tissues on which the fibrinogen is constantly deposited, loaded with acidic coagulating bodies and passing into the state of fibrin.*

This phenomenon of continuous deposition of fibrin in the tissues is made very apparent by the known fact that venous blood provides significantly less fibrin than arterial blood when it coagulates; the fibrin that disappears is that which has been deposited in the tissues.

The tissues therefore play, with respect to the blood in the process of continuous coagulation in the body, the same role as the rod which, agitating the blood in a vase, removes the fibrin which attaches itself to it as it is formed.

This phenomenon of coagulation is certainly not specific to animals; it must also take place in plant organisms, and, more generally, in all living organisms, these being always constituted by an organized mold, and any mold can only be born and grow by the formation, by means of its zymospores, of a gelatinous, sticky substance, which is deposited on the evolving elements. This substance which can be called hyaline matter (mycofibrin), and which is the formative protoplasm in the process of coagulating, is also the matter which has been called agglutinin; it therefore develops as well in bacterial cultures, formed of unorganized elements, as in organized beings.

IV. Nature and constitution of complement or alexin Mechanism of specific hemolysis of blood cells.

When the blood cells of one animal species are washed in salt water and injected into the blood of another animal, the serum of the latter acquires, after ten to twelve days, the property of rapidly hemolyzing, in vitro, in one or two hours, the blood cells of the same species as those injected. This serum from the animal, called sensitized, dissolves, as a general rule, only the blood cells of the sensitizing species. The phenomenon is therefore specific.

It has been deduced from this phenomenon that an immunizing antibody is formed in the blood of the sensitized animal, a lysine, also called sensitizing, resulting from a defense reaction of the organism and intended to rid it of the injected blood cells.

Sensitizing blood cells or antigens hemolyze in an hour or two at 38°C in the serum of the sensitized animal. They no longer dissolve if this serum is first heated for about 30 minutes at 57°C.

In this case, the phenomenon of hemolysis is suppressed because one of the agents that determine it, alexin, or complement, is destroyed. The study of the phenomenon of specific hemolysis is therefore inseparable from the study of one of the agents necessary to cause it, alexin, or complement.

Nature and constitution of complement or alexin.

It is generally accepted that alexin, or complement, is formed by the union of two substances that are easily separated by dialysis, or by diluting the serum ten times its volume with distilled water and passing a stream of carbonic acid through it. By either of these operations, one of the constituents, the middle part of the complement (mittel-

stück), precipitates; it is easily collected by centrifugation; this precipitate is soluble in salt water at 7.5 or 8 per 1000, and by adding, if necessary, a trace of sodium carbonate.

The clear liquid, the residue of the serum after centrifugation, constitutes the second portion of alexin, called the terminal portion of the complement (endstück).

These two portions, added separately to globules sensitized, do not hemolyze either one. If they are added simultaneously, the complement's power is reconstituted.

The middle part (mittelstück), heated for 30 minutes at 57°, loses its activity: it is thermolabile. The terminal part, heated to the same temperature, is not altered: it is thermostable. We see, from this presentation, that the two constituents of alexin are two very vaguely defined bodies and that the fact of their separation provides little information about its constitution.

From a long series of works, I concluded in 1914 **(1)**:

That alexin is a complex formed by the union of two bodies, one of which (middle part, mittelstück) is constituted by an albuminous body bound to soda soaps and the other by an albuminous body bound to cholesterol soaps (or ethers thereof). In these works, I admitted and took as my guiding idea the fact that alexin is constituted by the two substances indicated above and I sought to determine the constitution of each of them.

Currently, and thanks to a determinism of a completely different nature, I have been able to see that the determination I made in 1914, which was correct with regard to the albuminous substances contained in the middle and terminal portions of the complement, could not be correct with regard to the very constitution of alexin, because the guiding idea of the research was false. The fact that the serum divides, by dialysis, into two separately inactive and active parts when they are united, in no way signifies that alexin is a complex constituted by the union of these two parts; by giving the effect of the separation of the two substances the strict meaning that it entails, one must conclude only that one cannot act without the other, nothing more; it in no way authorizes one to conclude that a complex exists.

The determinism of the nature and constitution of alexin, or rather of the lysing action of complement, is much simpler than one could imagine.

The simplification results from knowledge of the nature of living organisms, knowledge of the development of molds and bacterial cultures, and the notions provided, subsequently, by the study of ferments and that of blood coagulation.

(1) *C. R. Acad, des Sciences, T. 158, 1914.*

Being in possession of this knowledge, the determination of the action lysing of the serum, called alexin by Buchner, can be done by posing the problem in the following way:

By dialyzing the serum, it is divided into two separately inactive parts; the middle part (mittelstück) is inactivated by heating to 57° or by the addition of a precipitate, such as tricalcium phosphate. We know, moreover, that:

1. If sensitized red blood cells are placed in contact with the middle portion, they fix a part of the active body that it contains.

2. That this middle portion, consisting of a portion of the serum globulins (the most easily precipitated), is active only by an infinitesimal part of its substance; the exhaustion of the active substance by sensitized globules does not remove, from the mass of the precipitated middle portion, a perceptible quantity of substance.

It is therefore a question of determining what this active substance is, of such reduced volume, inactivatable at 57°, and carried along by the precipitates. *Microscopic* examination of the middle part of the precipitated complement allows us to resolve the question; the active body is constituted by the zymospores of the serum, carried along by the precipitation of the globulins. These zymospores are carried along by all the fine precipitates, by the addition of tricalcium phosphate followed by centrifugation, for example. Examining the centrifugal precipitate, we find the zymospores there; *if the globulin precipitate, determined by carbonic acid or dialysis contains them*, it is because it acts like the other precipitates and carries them along; it carries them along even better than any other, because it is a precipitate of albumin combined with a soap; Their presence is easily observed in the precipitate spread on a glass slide and stained with dahlia.

The sensitized red blood cells, added to the redissolved middle part of the complement , act like another precipitate and act all the better because they have fixed a viscous and adhesive body: the sensitizer. The centrifugation to which the middle part must be subjected, to remove the red blood cells, has the effect of carrying the zymospores with them into the centrifugation pellet.

These are the same zymospores that constitute the serozyme. What is carried along, by precipitating the serum globulins, are the zymospores which, by their hydrolyzing action on another body, a lecithin (cytozyme), form the acid body that coagulates the fibrinogen (thrombin).

These zymospores are inactivated like the elements of the ferments by heating for 30 minutes at 57°. It is these and the other elements of the ferment that are inactivated by heat, *because an unheated, cultivated ferment multiplies; a ferment heated for 30 minutes at 57° no longer multiplies.*

We know that the middle part of the complement (mittelstück), being precipitated and remaining in this state and in contact with water containing less than 1/1000 of NaCl, only deteriorates slowly; we also know that the precipitate, redissolved in salt water at 7.5 per 1000, deteriorates very quickly (Brandt phenomenon). If we examine a precipitate dating back 24 hours under a microscope, we see that it contains many zymospores scattered or in small clusters; If we examine a solution of the same precipitate 24 hours old, we see that the zymospores have vegetated, formed germinative masses and even mycelial clusters as occurs in a solution of fibrinogen.

Therefore, if the solution of the precipitate becomes inactivated, it is because the zymospores evolve, transform and form hyaline matter which agglutinates them; if, in the unredissolved precipitate, the mittelstück remains intact, it is because the zymospores do not evolve there.

The active body, in the middle portion of the complement, is therefore constituted by the zymospores of the serum whose total mass is tiny compared to that of the globulins precipitated by carbonic acid or by dialysis; this active body being a ferment and being precisely that which forms thrombin, it exerts its effect by a fermentative action which is necessarily the same as that which determines the coagulation of the blood: a hydrolysis of the lecithins.

This tells us that the active substance of the terminal part of complement, the second constituent of alexin, is the same thermostable body that has been indicated as one of the constituents (cytozyme) of thrombin: that is, a lecithin. It is this body that is removed from serum or plasma by agitation with ether, the latter removing or profoundly altering both the coagulating power and the complementary power of the serum or the coagulation faculty of the plasma.

I therefore conclude:

1. That what Büchner called alexin is the product of a reaction that takes place in the blood, plasma, or serum, between the zymospores of the blood and the lecithins: it is the hydrolysis of the latter.

2. That the body possessing the lysing or bactericidal power in the serum is constituted neither by the hydrolysed body, lecithin, nor by the hydrolysing part, the zymospores,

but by one of the products of hydrolysis carried out between them, the acid fraction of hydrolyzed lecithins.

3. That the fibrin-ferment or thrombin is the group of two substances corresponding to Büchner's alexin, the serozyme being the middle part of the complement, the cytozyme its terminal part.

4. That the serum has a lysing or bactericidal power because the normal fixing body for the acid products of lecithin hydrolysis, fibrinogen, has been removed.

Indeed, the addition to the lysing serum of a fixing body replacing fibrinogen, globulin, for example, suppresses this lysing power: this is the mechanism of the known anti-complementary action of globulin.

It is therefore incorrect to say that the bactericidal action in serum observed by Büchner, or the lysing action that a serum can exert on blood cells, exists in normal blood plasma.

In the latter, the acid products of the hydrolysis of lecithins are immediately and constantly fixed by fibrinogen.

5. That the body which exerts the lysing action is the same as that which exerts the coagulating action; it is a body with an acid reaction to phthalein; it is obviously the portion of lecithin comprising glycerophosphoric acid and fatty acids.

Glycerophosphoric acid has a powerful hemolytic action; sensitized globules fix it more easily than non-sensitized globules. Fatty acids, particularly oleic acid, have an identical action, also acting preferentially on sensitized globules, a fact I have already made known **(1)**.

Von Liebermann concluded, following a series of studies, that alexine could be artificially reconstituted by a combination of alkaline soaps and globulin dissolved in a liquid whose saline constitution had to meet specific conditions. He concluded that this mixture is indeed a complement, because it is hemolytic and is inactivated by heat. This conclusion had few supporters.

This mixture is hemolytic because it contains a hemolytic body, soap, which does not allow any conclusion to be drawn with respect to serum or plasma, *in which soap does not exert a hemolytic function.*

The presence of globulin in this artificial supplement only has a lessening effect on the hemolytic power of the soap, because the globulin dissociates the alkaline soaps and these partially combine with it and precipitate it.

(1) *C. R. Acad. des Sciences, June 23, 1919.*

It is as a result of this reaction that the artificial supplement quickly becomes inactivated when heated, the reaction being accelerated by heat.

The preferential action that this artificial complement exerts on sensitized glo- bules is not greater than that of a fine emulsion of oleic acid. Von Liebermann's complement therefore does not present the essential characteristics of the lysing property of serum; the globulin that this experimenter believed necessary in the complement is, *on the contrary, an anticomplementary body*; it plays such a role, *even when it is added to a hemolytic serum, because it replaces, to a certain extent, the removed fibrinogen* and serves as a fixing body for the hemolytic acid bodies, as does a small quantity of added alkali. In normal serum left to itself, the globulin exerts this fixation progressively and becomes, because of this, more and more sensitive to the various agents that precipitate it.

Mechanism of specific hemolysis of blood cells.

A sensitized red blood cell, placed in vitro in contact with fresh complement, is hemolyzed.

We have determined the nature and role of the complement elements. We know that the active element of its middle part (mittelstück) is the serum zymospore, and that it also constitutes the ferment called serozyme; the active element of the terminal part (endstück) is the lecithin it contains; these two parts cannot act separately, because they are both agents of a hydrolysis phenomenon; one (mittelstück, serozyme) is the hydrolyzing ferment constituted by the serum zymospores; the other (endstück, cytozyme or lecithin) is the hydrolyzed body.

The product of hydrolysis is a body with an acid reaction to phthalein, which determines either the coagulation of fibrinogen (thrombin), or the hemolysis of heterogeneous non-sensitized globules (natural hemolytic property of sera), or the hemolysis of sensitized red blood cells (specific hemolysis). To understand the mechanism of the hemolysis phenomenon, it is therefore still necessary to determine:

1. What phenomena cause the antigen red blood cell to appear in the sensitized organism to cause the formation of the sensitizer?

2. The nature and properties of the sensitizer.
3. Why do antigen red blood cells bind the sensitizer and are the only ones to bind it?
4. What reactions take place between the sensitized red blood cell and the complement elements brought into contact with it?

5. Why does the red blood cell become disorganized and what is the mechanism of this disorganization?

Causes of the specificity of the hemolytic power of sensitized sera.

A blood cell is an element formed by the mold constituting the animal organism; this mold is specific and differs from one species to another. This explains why certain elements of a specific mold, developed in the blood of an animal, *react only with the elements of this same mold,* just as the products developed by the zymospores of a heterogeneous mold, the typhus agent for example, *react exclusively with the bacterial elements of this agent.*

This specificity, establishing that the *elements of the organic mold of an animal only react on the elements originating from this same mold,* it follows that specific hemolysis consists, not of a destruction, in the strict sense of the word, but of an evolution or transformation of the red blood cells, because *elements of a mold do not kill other elements of the same species.*

Destiny of red blood cells introduced into the blood of another animal species. Mechanism of the development of the sensitizing power in the blood of the prepared animal.

Red blood cells, being living elements of an animal's organic mold, evolve like the elements of any other mold, for example, a pathogenic mold, when they are transported into the organism of another animal. *Leaving the organism to which they belong, the red blood cells no longer differ, for another animal, from the elements of any heterogeneous mold. Transported to another environment, the red blood cells therefore evolve like any mold: they lose their organized form and their elements first develop a bacterial culture in the injected organism*, *then, after a certain time, ten to fifteen days, they pass into a stable mycelial state in the tissues and develop a long-lasting mycosis.*

Like any mold, the injected red blood cells develop, in the blood of the prepared animal, bacterial elements, zymospores, and the elements necessary for the development of the formed elements: *the hyaline matter and the body(ies) forming the basophilic (sensitizing) protoplasm.* Note that the sensitizing hyaline matter only develops in large quantities in the blood of the injected animal from the eighth to the twelfth day.

From this development of the injected red blood cells, I conclude firstly:

1. That the disappearance of heterogeneous red blood cells in the blood of the prepa red animal *does not result from their destruction, but from their natural evolution; they lose their organization, but the mold that constitutes them remains alive and evolves.*

2. That this disappearance and the correlative formation, in the blood of the prepared animal, of the bacterial and other elements of the preparing mold, have nothing in common with a phenomenon of defense of the organism. The sensitizer is a constituent element of the antigenic mold with no original relationship to the sensitized organism.

I must point out here that these conclusions are drawn from material facts and that any hypothesis is excluded. Indeed, the injection of blood, liver pulp, brain, or any other organ into an animal of another species, develops in its organism the elements of the injected mold that are easily found fifteen days, a month, or two months later, by culturing the pulp of the tissues of the injected animal on agar. This results from experiments carried out in considerable numbers, by the hundreds, and is said to oppose it to the following reasoning:

The prepared animal serum acquires, after ten to fifteen days, the property, which it retains despite heating at 57° for thirty minutes, of making the globules of the sensitizing species easily hemolyzable by a complement of any origin (Gengou). From this phenomenon, it has been deduced:

1. That there exists in the serum of the prepared animal a body called a hemolytic sensitizer (Gengou) which binds exclusively to the globules of the sensitizing species and makes them capable of being hemolyzed.

2. That this sensitizer is an immunizing antibody formed by a defense reaction of the organism.

The sensitizing action of the prepared serum is based on a material fact, therefore beyond doubt. As for the conclusion which transforms this fact into an immune reaction, it is not based on any material fact and does not result from any direct or indirect demonstration; it is a pure hypothesis demonstrated to be inaccurate by the facts presented so far and by those which follow.

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Nature and properties of the sensitizing substance
Causes of its specific fixation on the antigen red blood cell.

A red blood cell freshly removed from the antigen organism being placed in the serum of the prepared animal (heated to 58° for 20 or 25 minutes) acquires a special property: being removed from this serum, then placed, in vitro, in contact with fresh complement from another animal species, it hemolyzes there, while another fresh red blood cell, which has not undergone the prior treatment, does not hemolyze there.

Quite often, fresh antigen red blood cells introduced in vitro into the heated serum of the prepared animal quickly agglutinate and fall to the bottom of the tube.

These fresh red blood cells therefore remove from the serum of the prepared animal a substance that agglutinates them and gives them the property of energetically fixing the substance that possesses the hemolytic power in the serum, alexin. We have seen that this hemolytic substance is the acid portion of the lecithins hydrolyzed by the zymospores of the serum (mittelstück or serozyme).

The substance that confers these properties to the antigen red blood cells by attaching itself to them, and which is called sensitizer or amboceptor, is the hyaline matter of the antigen culture developed in the blood of the prepared animal; it attaches itself to the antigen red blood cells and not to others, because they have, with the formed elements of the culture, the necessary specific link; they belong to the same original mold.

There are not two substances, one sensitizing and the other agglutinating; there is only one, the hyaline matter, possessing these two properties.

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This hyaline substance is a globulin; in the prepared animal serum, it is completely precipitated by sodium sulfate with normal globulins; it is deposited on bacterial elements, in in vitro cultures, in the form of a gelatinous, sticky, adhesive substance, like fibrin. It therefore has the appearance of fibrin and comes from the coagulation of a globulin, just as animal fibrin comes from the coagulation of fibrinogen, which is also a body of the globulin series and the most precipitable of them.

Added to this identity of nature is the identity of destination of hyaline matter and fibrin. Hyaline matter is the substance which, by coagulating on bacterial elements, causes their agglomeration and the development of mold. It has been demonstrated elsewhere, regarding the coagulation of fibrinogen, that the destiny of fibrin is the formation of the vegetative elements of organic mold.

Fibrin is formed by fixation, on fibrinogen, of the acid portion of hydrolyzed lecithins. All globulins have, like fibrinogen, a powerful affinity for this acid portion of lecithins; hyaline or sensitizing matter, which is a globulin, therefore also possesses this affinity.

This affinity of the hyaline substance for the acid portion of lecithins and fatty acids can be made apparent, as has already been done for fibrinogen, by introducing into the liquids opposite it substances which have a lesser, equal, or greater affinity than its own for these bodies and which replace it in fixing them. In this case, hemolysis is hindered, greatly delayed, or completely prevented. As with the coagulation of fibrinogen, the addition of a small quantity of caustic soda to the mixture of red blood cells and complement completely prevents hemolysis, because the soda fixes the acids released

by hydrolysis; hemolysis does not take place as long as there remains soda unsaturated by these acids; it begins to take place only when saturation has taken place; a solution of serum globulin, added to the mixture, exerts the same effect for the same reason.

Reactions that take place between the sensitized red blood cell and the complement elements brought into contact with it.

These reactions become very apparent now that the constituent elements of complement and the properties of the sensitizing substance are known.

The red blood cell is sensitized because a thin layer of hyaline material is deposited on its surface, possessing a great affinity for the acid portion of the lecithins in the serum; because of this affinity, it fixes, as it is released, the acid product of the hydrolysis of the lecithins carried out by the zymospores (mittelstück, serozyme). Now, the acid products of this hydrolysis (glycerophosphoric acid and fatty acids) possess a powerful hemolytic power.

The proof of their fixation on the red blood cell lies in the suppression of hemolysis when a body, alkali or globulin, is added to the elements present, which monopolizes the released acid portion and thus prevents its fixation on the red blood cell.

I recall that I had already been able to establish, in 1919 **(1)**, by very different means, that the sensitization of red blood cells consists of the fixation of a body which gives them a powerful affinity for fatty acids and that their hemolysis results from the action of the latter.

When the reaction is carried out by bringing the sensitized red blood cells into contact, first with the mittelstück dissolved in salt water, then with the endstück, the hemolytic action takes place in the following way: the sensitized red blood cells act on the mittelstück solution as would any other precipitate, carrying with them the active element, the zymospores (serozyme), in the centrifugation that the emulsion undergoes to remove them. By returning the red blood cells to the endstück, the zymospores are introduced with them.

The endstück therefore recovers the element that it lacked, the zymospores or serozyme, or that it possessed in insufficient quantity to be able to function as a complement, that is to say for the hydrolysis of the lecithins to be able to take place there.

(1) *Acad. des Sc., page 1283, t. 168.*

Microscopic study and mechanism of the destruction of red blood cell organization in the hemolysis reaction.

One last point to determine is the mechanism of red blood cell destruction; to study it, I turned to turtle red blood cells; I injected a rabbit three times in a row, two days apart, with 1 to 2 cubic centimeters of turtle red blood cells.

Twelve days after the last injection, the serum from this rabbit, added with turtle red blood cells, hemolyzed them. For microscopic examination of the phenomenon of hemolysis, the blood elements must be stained with dahlia. This staining can first be done in a test tube, the red blood cells centrifuged, and then added to the prepared serum. Alternatively, simply add a little dahlia solution to the mixture. But it is better to stain the red blood cells before mixing, because in this case, we are certain that only the turtle leukocytes are stained, which avoids confusing them with the germinal masses of the rabbit serum.

Under these conditions, the nucleus of the red blood cells is colored dark blue and the mycelial framework of the peripheral zone in very pale blue, here is what we observe: the attack of the red blood cells is not immediate, but, quite quickly, we see a hole, a window, appear in their hyaline peripheral zone, then one or more others after; all widen while the entire peripheral mass becomes more and more translucent; we see that it dissolves; it soon ceases to be visible and the nucleus of the hematic, released, becomes floating in the liquid and remains brightly colored; however the blue tint of certain nuclei fades.

If care has been taken to choose a field in which several turtle leukocytes (which are very large) exist, we see the nucleus of the red blood cell come into contact with one of them, then gradually penetrate and merge with it entirely despite its large size compared to that of the leukocyte (diameter equal to one-half or two-thirds that of the leukocyte). By scanning the preparation, we see the same operation taking place in a large number of leukocytes **(1)**.

This observation shows that the hyaloplasma of the red blood cells is dissolved by the prepared rabbit serum, but that their basophilic nuclei are not affected and come to constitute large basophilic masses, that is, *germinative masses by fusing with the leukocytes.*

(1) *This phenomenon is the one that led to the designation of certain leukocytes by the name of macrophages; these leukocytes encompass the elements of neighboring cells in the process of disorganization; they are often very large because their volume increases through the contribution of new elements; they are not macrophages; they are leukocytes, simply belonging to the specific organic mold, they agglomerate any free element of it; they do not digest these elements; these are simply incorporated to complete the constitution of the germinative mass which, sufficiently provided with basophilic protoplasm, will enter into germination.*

The disorganization of the red blood cell by a prepared serum therefore does not correspond to destruction, but, on the contrary, to an evolution with a view to the multiplication of the mold that constitutes it.

This phenomenon is the confirmation of the one we have already observed when studying the action of an antitoxin on a toxin. The diphtheria antitoxin added to the toxin does not exert any destructive action on it; on the contrary, we find that, in this mixture, a culture of the diphtheria agent develops, a fact which, moreover, also occurs in the toxin alone.

The study of the evolution of red blood cells during specific hemolysis leads us to analogous observations.

To study the action of specific hemolytic sera, I prepared a rabbit by several successive injections of guinea pig blood cells. guinea pig. The prepared rabbit serum was mixed with nine times its volume of distilled water, then precipitated with carbonic acid to study respectively the hemolytic properties of the precipitate and the remaining liquid **(1).** The precipitate was redissolved in a quantity of salt water (7.5 per 1000) equal to the volume of the original hemolyzing serum; then, to 0 cc. 2 of guinea pig globules were added a cubic centimeter of salt water, 0 cc. 2 of normal rabbit serum and 0 cc. 2 of the solution of the precipitate of hemolytic rabbit serum (sensitizer). The tube was heated to an oven at 380 for one hour. At the end of this time, only partial hemolysis was noted. But, examining the liquid under a microscope, I noted with the greatest astonishment that all the blood globules had emitted mycelial filaments, some already branched, others ending in a spore.

The blood corpuscles had entered into vegetation very quickly. They had almost all become germinative masses which were entering into vegetation. The shape of the mycelial filaments emitted by the corpuscles, their ramifications, the dilations they carried along their path, the terminal spores carried by some of them left no doubt about the nature of the phenomenon and the role of germinative mass played by the corpuscles. Plate 121 (Fig. 1 to 4) shows the photograph of the various regions of a preparation. In figures 1 and 2 we note that a fairly large basophilic mass has formed on the periphery of the corpuscles or in their interior and that it is this which emits the germinative filament; some of these masses have become free with their germinative filament, but they retain the trace of their origin by shreds of the globular framework which remain adherent to them.

(1) *The precipitate contains, at least in large part, the sensitizer.*

The blood cell is therefore destroyed because it undergoes the same evolution as the bacterial elements in a culture; its elements dislocate and then fuse to form a germinative mass; in the current case, this has reached the germination stage.

This vegetation of blood cells is not surprising since they are mold elements containing spores, basophilic and acidophilic elements capable of evolving to reproduce the original mold. From this evolution of blood cells, we can draw the following conclusions:

1. Blood cells from one animal species injected into another species are capable, even if they are free of white blood cells or other elements, of vegetating themselves in the inoculated organism and of producing the mycosis of the antigen organic mold.

2. A blood effusion totally devoid of foreign germs and even devoid of white blood cells is capable of becoming purulent by the sole power of vegetation of the red blood cells.

These facts will serve as a basis for explaining the phenomena of specific hemolysis or cytolysis. Blood cells injected into an animal develop in it the mycosis of the organic mold specific to the antigen animal. The serum of the prepared animal therefore becomes agglutinating (as in the case of bacterial antigens) for the elements of the injected mold, the blood cells. In addition, the other elements that develop in the case of inoculation with pathogenic germs develop in the blood and humors of the prepared animal; *the blood will contain, in particular, the various species of acidophilic and basophilic spores or zymospores as well as the hyaline matter or agglutinin of the injected mold.*

Whether it is a prepared anti-red blood cell serum or a serum called antibacterial, we obtain the same result; the serum does not exert a destructive action; it only exerts an evolutionary action on the antigen.

CONCLUSIONS

I summarize this study with the following conclusions, separating the phenomenon of sensitization from the phenomenon of hemolysis itself:

Sensitization.

1. Animal red blood cells are organized elements of their specific organic moiety.

2. Red blood cells, introduced into the blood of another animal, lose their organization to transform into a bacterial culture of the antigenic organic moiety. This culture contains, in addition to the usual figurative elements, the elements constituting the hyaline substance.

3. The hyaline substance, contained in the serum of the prepared animal, is the substance that causes the agglutination (agglutinin) of the elements for the formation of germinal masses; fresh antigenic red blood cells fix this hyaline matter because of their specific bond with the elements of the culture; it is therefore the sensitizer at the same time as the agglutinin.

4. The hyaline matter, fibrin of cultures of heterogeneous molds (sensitizer, amboceptor, agglutinin), possesses, like the fibrin of animal blood, a strong affinity for the acid portion of lecithins and for fatty acids; it owes its sensitizing property to this affinity.

5. The agglutinating and sensitizing action of the hyaline matter is specific, because it is developed by a mold and the evolutionary reactions of the elements of the molds only take place between those which belong to the same species; the elements formed by the specific organic mold of an animal can only react with those which come from this same mold.

6. The sensitizer is the substance that fixes and attracts, on the surface of the red blood cell, the hemolytic acid bodies that disorganize the red blood cell; this disorganizing action does not correspond to an action harmful to the mold that constitutes the red blood cell, but on the contrary to a favorable evolutionary action; it is therefore the organization of the red blood cell alone that is destroyed.

Specific hemolysis.

1. Hemolysis is specific because sensitization is specific for the reason indicated above (5°).
2. Sensitizing red blood cells, placed in the sensitized serum, fix hyaline matter like the other elements of their mold.

3. From this fixation of hyaline, sticky, and adhesive matter, results the agglutination of the red blood cells and their sensitization.

4. Hemolysis results from the fixation, on sensitized red blood cells, of the acidic products of lecithin hemolysis.

5. These products fix on the sensitized red blood cells because the sensitizing hyaline matter has a strong affinity for them.

Significance of the preceding notions relative to the mechanism of immunity.

The analysis of the mechanism of sensitization and specific hemolysis leads us to important conclusions relative to the knowledge of immunity.

Sensitizers are considered to be immunizing antibodies, formed by the animal organism and by a defense reaction.

However, it follows from the preceding study that the hemolytic sensitizer is the hyaline material *formed by the elements of the sensitizing mold culture* and not by the organism of the prepared animal.

The sensitizer is therefore not an immunizing antibody and its formation has nothing in common with a defense reaction of the organism.

Moreover, let us analyze the phenomenon from the point of view of this defense reaction. We see:

1. That red blood cells, introduced for the first time into the blood of an animal, do not persist there in their form and that they easily disorganize to give rise to a bacterial culture. There is therefore no need for the formation of a particular protective body to disorganize them in the blood since they disorganize spontaneously.

2. That the presumed antibody, the sensitizer, only appears clearly in the blood at the time of the passage of the bacterial culture to the mycelial period, after ten to twelve days, and that this appearance coincides with the passage of the culture to the mycelial state and not with a need to destroy the red blood cells, this having been carried out for a long time.

3. *That, despite the presence of this presumed immunizing antibody, the injected mold persists in the prepared organism from where it can be extracted after a month or two, proof that it is in no way destroyed or affected by this antibody.*

4. That the reaction which, in the blood, follows the injection of an antigen, in no way has the effect of protecting against its possible subsequent penetration, since this can, on the contrary, provoke anaphylactic shock.

5. That this antibody does indeed exert a modifying action on the antigen, but that it is in no way a reaction of destruction or arrest of its development; on the contrary, it is an evolutionary reaction favoring this development.

Other equally convincing facts show that the sensitizer in no way has the effect of provoking the lysis and destruction of the sensitized antigen. The sensitizer is the same body as agglutinin; it is the hyaline matter of the mold. In typhoid fever, the appearance of the agglutinating power is early, since it is this which serves to establish a relatively sure diagnosis because of the link of specificity which exists between the typhoid bacterial elements and the agglutinating hyaline matter that they themselves form.

Why do the bacilli continue to develop and proliferate in the body in this case, since the antibody has been formed? Why does the patient's condition not at least improve from the moment the presence of the agglutinating hyaline matter, which is also a sensitizer, is noted?

There is not the slightest doubt that no immunizing antibody is formed in this case.

We can therefore conclude:

1. That the sensitizers, lysines, are bodies formed by the antigen itself to ensure its development and multiplication.

2. That these bodies have nothing to do with a defense process of the organism.

3. That the antigen disappears, in the bacterial state, only to change form and pass into the stable mycelial state.

4. That the fact of the passage and the long persistence of the antigen in the mycelial state in the organism demonstrates that no defense reaction intervenes to destroy it.

Mechanism of natural hemolysis by serums or by organ juices.

In normal blood, red blood cells do not hemolyze, despite the existence of the constant physiological phenomenon of lecithin hydrolysis because, once detached, the acid portion of the latter is fixed by a body that has a strong affinity for it: fibrinogen.

Red blood cells also do not hemolyze in their own serum because globulins have a great affinity for the acid portion of lecithins. However, over time, red blood cells end up hemolyzing in their own serum, the capacity for fixing globulins of which diminishes, while the phenomenon of lecithin hydrolysis continues until the latter are exhausted; at a given moment, the fixing capacity of red blood cells equals that of globulins, and hemolysis results.

The natural hemolysis of heterogeneous or autogenous red blood cells by a serum is carried out, like the hemolysis of a sensitized red blood cell, by alexin or complement, that is to say by the acid portion of the lecithin molecule hydrolyzed by the zymospores of the serum; natural hemolysins in organ extracts are made by the same body.

If we try to dissociate the complementary property from the natural hemolytic property, we see that it is impossible to achieve this and we note that the identity between these two properties is so perfect that they merge; in fact:

1. Heating progressively alters, to the same extent, these two properties; they disappear together **(1)**.

2. The addition of globulin to serum alters both properties simultaneously and to the same extent; the addition of a small quantity of caustic soda has an identical effect.

3. Dialysis alters both properties simultaneously; the natural hemolytic power is eliminated like the complementary power by the separation of the globulins and serum albumin; it reappears by the reunion of the two parts.

The juices obtained by grinding organs or tissues with salt water often exhibit hemolytic power; this is of the same nature as that of serums and is subject to the same influences. These tissue juices (cytozyme) promote the coagulation of blood or plasma through the lecithin they contain (Bordet). They also contain zymospores from the ground tissue that can hydrolyze the lecithin.

The hemolytic power of such a juice therefore differs in no way from that of a serum; it does not contain hemolysin; These are the normal elements of lecithin hydrolysis which act here, and they act with all the more ease as the juice contains very few bodies (globulins) capable of fixing the hemolysing acid bodies. From these facts it follows:

1. That red blood cells can hemolyze even in their own serum.

2. That if red blood cells do not hemolyze normally in the blood, it is because the liquid which surrounds them contains substances more eager for hemolysing bodies than they are: fibrinogen and globulins.

3. That if the most energetically binding substance, fibrinogen, is removed from the plasma, the remaining liquid (serum) can acquire a hemolytic power for heterogeneous red blood cells *that plasma does not possess.*

4. *That the body which produces the natural hemolysis of heterogeneous red blood cells, in a serum, is the same as that which causes the coagulation of fibrinogen: the acid portion of the hydrolyzed lecithin molecule.*

5. That the hemolytic power of a serum for heterogeneous red blood cells, or even for red blood cells of the same species, comes from the fact that their transport in this serum places them in conditions of fixation of acid bodies different from those to which they are adapted.

(1) *Except in a single case, that of dog serum in which a phenomenon occurs for which I have not seen the explanation: In this serum, the complementary property disappears by heating for 30 minutes at 58°; on the contrary, the value of natural hemolytic power increases with temperature and becomes maximum around 80 or 90°. This hemolytic power exists for the dog's own red blood cells.*

Indeed, in this new environment, there is a change in the proportion of acid bodies formed and in the ratio between the fixation power of the red blood cells and that of the other fixing bodies of their own serum.

6. The hemolytic properties of sera for heterogeneous red blood cells are not due to hemolysins or natural immunizing bodies. These hemolysins do not exist. There is only a hemolyzing property, coinciding with a change in the conditions of the normal red blood cell environment.

These hemolytic properties of sera come mainly from the absence of fibrinogen in these fluids. *Indeed, the restitution of the removed fibrinogen eliminates them.* They do not exist in plasma or normal blood.

V. Study of the mechanism of the agglutination phenomenon.

The injection of an antigen substance into the blood of an animal develops the power to agglutinate the elements constituting this substance; from this agglutinating power, the formation, in the blood, of a specific agglutinating substance called *agglutinin* has been deduced.

It has just been demonstrated above that this agglutinin is confused with the body called sensitizer, and that the two functions, agglutinating and sensitizing, are exercised by a single substance, a hyaline, viscous, adhesive material, analogous to fibrin and which, like it, comes from a globulin; this substance is formed by the antigen itself and results from the chemical activity of its zymospores, just as the viscous hyaline material of the organic mold of an animal, fibrin, is formed by the chemical activity of the zymospores of the blood.

The phenomenon of agglutination and the formation of the substance that determines it exist in in vitro cultures. We see that bacterial elements agglomerate into large clumps in cultures, in those of the typhus bacillus, for example, when they are several days old. These clumps are formed because the culture develops hyaline substance that is deposited on the bacterial elements and causes them to stick together when they touch.

The formation and coagulation of the hyaline matter, then the agglomeration or clumping of the bacterial elements on which it is deposited, is an important phenomenon, a phase in the development of the bacterial elements of molds.

The hyaline matter, which is agglutinin and at the same time the sensitizer or lysine, is formed by the antigen itself and *not by the animal organism*; it has nothing in common with a phenomenon of the organism's defense.

Agglutination, by the hyaline matter formed in a culture, is one of the first phenomena that I had the opportunity to observe at the beginning of the research that is the subject of this work.

I was able to see, in fact, the phenomenon of agglutination by the hyaline matter and the formation of germinal masses taking place before my eyes.

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Having observed numerous bacillary elements in the juice obtained by scraping breast cancer tissue, I made a culture. After twenty-four hours, the bacillus observed the day before was teeming in it. Having introduced a drop of this culture between slide and coverslip with a little dahlia solution, I saw that it contained large basophilic masses, each consisting of the agglomeration of spherical secondary masses 15 to 20 μm in diameter. Around these masses was deposited a thick layer (20 to 30 μm) of *viscous* hyaline matter, non-basophilic and barely tinted a very pale purplish blue; I say that this substance was viscous because this property manifested itself with remarkable clarity.

In the field of vision, I saw numerous bacilli moving, not by movements of the liquid and not thanks to their own mobility; they were carried from one end of the field to another by a zymospore that I saw attached to one of their ends (sometimes in pairs, one at each end), and whose jerky movements due to its Brownian motion I clearly distinguished.

In its movement, each bacillus, moved by this zymospore, simultaneously performed lateral oscillating movements analogous to those of a boat whose oarsman would alternately operate the two oars.

From time to time, a bacillus thus carried along would strike the hyaline matter with its end, which remained attached to it; it would not detach itself, despite the rather lively lateral oscillations that the motor zymospore at the other end continued to give it. After seeing the same thing happen several times, I could no longer doubt that this hyaline matter was viscous and very adhesive. Continuing to observe the same field of vision, I saw that after three hours the bacilli attached to the periphery of the hyaline substance had penetrated it and had come into contact with the basophilic mass, one of them having even partially penetrated it, from which it still emerged by half its length. Then examining other fields, I saw masses of hyaline matter of all sizes containing bacilli, either still unfused or in the process of fusing; finally, certain uniform and totally basophilic masses emitted very long, strongly basophilic mycelial filaments at the ends of which bacilli, identical to those moving in the preparation, were in the process of detaching themselves.

The preparation thus showed the entire evolution of the bacterial elements, the coagulation of the hyaline matter, its deposition on the elements and its agglutinating role.

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It also showed that agglutination or agglomeration was favored or even caused, in the culture, by the movement imparted to the elements by the zymospores that attach to them: they impart this movement even to the shapeless mycelial debris to which they attach themselves.

The observation of the existence of viscous or agglutinating matter in bacterial cultures is quite easy. A drop of a bacterial culture a few days old is placed between slide and coverslip, with a little methylene blue or dahlia solution. The preparation almost always contains fairly large bacterial clumps, some immobile, others carried along by the currents of the liquid. In each clump, we note that many bacterial elements have no point of contact with the others and that they are nevertheless intimately united with the mass. This arrangement has been represented by a drawing (Fig. 4, pl. 122) in which we see several masses formed of elements included in an invisible gelatinous substance but firmly held by it.

Certain elements, such as bacillus A which touch the mass only by one end, are animated by an oscillating movement by the displacements of the liquid, but remain adherent to the mass. Other times, it is a whole group of elements (E), which is animated by swinging and remains fixed to its mass (D). We can also see three spores (H) joined by the same fibrinous filament to the main mass (B) oscillating vigorously, the most distant one describing an area of a circle whose center is the point of attachment of the fibrinous filament.

I attempted to photograph these agglutinated masses; two are shown in the photographs of Figures 1 and 2, plate 122. The agglutinating hyaline matter was stained clearly enough to be visible in Figure 1, where it forms very apparent shadows; these are, as in Figure 2, masses moving entirely within the liquid, the elements of which are firmly held together by the hyaline matter; impacts on the lamella dis- place the mass, but do not change the relationships between the elements; those which are distant from the mass, such as the A spores (Figs. 1 and 2), rigorously maintain their position regardless of the movement of the mass.

In certain cultures, the hyaline matter is very barely visible; however, with strong staining, it can be distinguished around the masses and often in groups of only two or three elements (Fig. 3, plate 122).

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Thus, direct observation of bacterial cultures in vitro shows that the agglutinating hyaline material is formed by the bacterial agent (or more generally by antigen **(1)**)

itself. This hyaline matter is formed only in very small quantities in in vitro cultures in broth not rich in albuminoid materials; in these cultures, only an extremely thin and almost invisible layer is deposited on the bacterial elements; it is for this reason that these cultures form germinative masses with difficulty and only of small size. The formation of hyaline matter is much more apparent in cultures on gelatin and agar. The richness in albuminous materials is the condition for the abundance of its formation.

The observed facts imply the following conclusions:

1. The agglutinin of an antigen is the viscous hyaline matter that it itself forms in the blood.

2. This hyaline matter or agglutinin is also well formed by the antigen in its in vitro culture, provided that it is provided with the necessary albuminous materials.

3. Hyaline matter is a necessary substance, formed for the development of mold.

4. The phenomenon of agglutination exists both in in vitro cultures and in the animal organism; it represents an essential phase in the development or evolution of the bacterial elements of molds.

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VI. Mechanism of the Precipitation Phenomenon.

When an albuminous substance is injected into an animal for several days in a row, its serum acquires, after ten to fifteen days, the property of causing a precipitate or flocculation in the solution of the antigen substance when it is added in small quantities to it. The serum is said to have acquired the precipitating property and this results from the formation, in the blood of the prepared animal, of an immunizing antibody called precipitin. The mechanism of this phenomenon is determined by the following facts:

1. The injected antigen is always a formation of the organic mold specific to an animal or plant. It always contains formed elements capable of reproducing it in its different forms, mycelial or bacterial.

2. The organic mold constituting the antigen develops its bacterial culture in the organism into which it is injected.

3. This culture passes into a stable mycelial state after twelve to fifteen days, thus, establishing a permanent mycosis in the injected organism.

4. The prepared serum possessing the precipitating property contains spores, zymospores, and the hyaline matter (as well as its formative elements) of the antigen mold.

(1) *Every antigen is a body emanating from an animal or a plant, that is to say from an organized mold; every antigen passes into the bacterial state in the animal organism into which it is injected.*

5. The organic matter, recently derived from the animal, deteriorates slowly because its formed elements evolve and reconstitute a bacterial culture of the ancestral type of the mold; when the elements of this constituted culture are added to the organic matter as soon as it leaves the organism, it deteriorates more quickly.

6. Adding serum from the blood of an animal into which it has been injected to the solution of an antigen amounts to adding to this antigen the elements of the bacterial culture of its ancestral organic mold.

Horse serum, collected aseptically and left in a test tube, precipitates and flocculates naturally after a certain time. If, as soon as it leaves the vessels, a drop of cloudy horse serum that is several days old is added, it flocculates more quickly. It will also flocculate more quickly if serum from an animal sensitized by the horse serum is added.

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If we examine the precipitate and flakes that appear in a serum to which a drop of precipitating serum has been added, we see that they are formed either from clusters of spores or very small germinative masses emitting a sinuous mycelial filament carrying zymospores, or from agglomerated bacillary elements, or from mycelial colonies of which certain filaments are in the process of segmenting into bacterial elements.

Figs. 5 and 6 of Plate 122 show two types of clusters which constitute the granulations of the precipitate and the flakes in horse serum to which a minimal quantity (one drop per 2 cubic centimeters) of precipitating serum has been added. The elements of characteristic shape of Figure 5 are found in horse serum which is left to flocculate naturally; they are easily recognizable, being either chains of coccobacilli, or chains of diplococci, when each coccobacillus has segmented into two spherical granulations.

The precipitation or flocculation of the antigen therefore consists of the formation of bacterial and mycelial elements of the organic mold that constitutes it.

The cause of this flocculation is the contribution of the two elements contained in the serum precipitating the hyaline matter of the antigen and the formed elements of the antigen culture, zymospores, spores, and often even germinal masses of various sizes. The hyaline matter, an agglutinating substance (agglutinin), determines the agglomeration of the formed elements contained in the antigen solution, that is, the formation of germinal masses that form mycelial colonies through their development. The spores in the solution also germinate and emit a mycelial filament that forms zymospores or spores.

It is by this mechanism that a precipitating serum acts on a filtered bacterial culture. The filtrate contains reproductive figurative elements, the zymospores of the culture. The addition of precipitating serum, which is at the same time agglutinating due to the

hyaline matter it contains, causes the zymospores to clump together into germinative masses that develop. *The culture filtrate, or toxin, becomes cloudy, that is, precipitates and flocculates spontaneously as well, but much more slowly;* the mechanism of development is the same, but here the hyaline matter is elaborated in the filtrate or toxin by the zymospores.

It is known that injecting cow's milk into an animal gives its serum the property of coagulating cow's casein. The injection of cow's milk (containing spores and zymospores) causes the development of a bacterial culture and then of the permanent mycosis of the organic mold of the cow.

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The precipitating serum contains hyaline matter and formed elements which, when added to cow's milk, cause active vegetation of the formed elements of the organic mold of the cow and the production of acid bodies which attach to the casein and coagulate it. Bacterial cultures of many mold species coagulate the milk by the same process.

It is known that some experimenters have been able to obtain precipitins by injecting animals with substances extracted from plants (Phytoprecipitins).

This fact is not exceptional, since the most widespread bacterial species are a vegetative form of the organic mold of plants. Indeed, the diphtheria, typhus, and paratyphoid bacilli, for example, are respectively the bacterial forms of the organic molds of barley, corn, wheat, and rye.

Plants therefore also produce agglutinins, since these are the same bodies that constitute precipitins.

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VII. Mechanism of the Complement Fixation Phenomenon.

The explanation of the mechanism of this phenomenon is made simple by knowledge of the facts presented previously. The active body of complement, or alexin, is the acid fraction of the lecithin molecule, released in the hydrolysis of the latter by the zymospores of the blood or serum (serozyme). It has been demonstrated that the released acid bodies bind, in the blood or plasma, to fibrinogen; in the serum, they gradually bind to the globulins.

If a material as avid for these bodies as the globulins is introduced into the serum, it will bind some of them; if it is much more avid than them, the material will bind almost all of them. Complement fixation is therefore the binding of the active acid bodies that result from the hydrolysis of the lecithins.

If a very small quantity of sodium hydroxide is introduced into the complement, it will fix the acidic bodies and prevent their transport to the globulins, just as it prevents it even on a very avid substance, fibrinogen; caustic soda therefore fixes the complement.

If sensitized red blood cells are introduced into the complement, the hyaline matter they have carried, more avid for acidic bodies than the globulins, will fix them, that is, the complement in their place, and there will be total hemolysis. But if the proportion of fixing globulin in the complement is increased by adding the globulin removed from another portion of serum, the hyaline matter will be inferior and will no longer fix a sufficient quantity of acid for hemolysis to occur, or, again, the hemolysis will be weak and delayed.

If, instead of sensitized red blood cells, we introduce into the complement another sensitized antigen, it will fix the acid bodies of the complement in the same way. Two hours or more later, this mixture will have lost a notable quantity of acid bodies fixed on the sensitized antigen and a corresponding portion of hydrolyzed lecithins. If, at this time, we add further sensitized red blood cells to the mixture of complement and sensitized antigen, it is in the following conditions:

1. The intensity of the hydrolysis phenomenon and the formation of acid bodies has diminished.

2. The sensitized antigen has fixed acid bodies and is still capable of fixing them again.
3. The newly introduced sensitized red blood cells are capable of fixing these bodies as well.
4. The complement globulins have retained their ability to fix.

The result of the reaction therefore depends, firstly, on the quantity of hyaline matter fixed on the sensitized antigen, on the quantity of acid bodies that this matter has fixed, and on its remaining fixing capacity. If there is no hemolysis of the sensitized red blood cells, it becomes certain that a significant portion of the hyaline matter of the antigen had been introduced into the complement, that is, that the antigen was truly sensitized.

But if hemolysis occurs to a small degree, or even if it is total, it is very difficult to state with certainty that the result is due to the absence of sensitization of the antigen, because, in this case, the reaction depends on the respective values of the four factors listed above.

If, for example, the antigen is weakly sensitized and the red blood cells are, on the other hand, hypersensitized, total hemolysis may result and falsely conclude that the antigen was not sensitized.

We see, on the other hand, the considerable influence exerted by the respective quantity of each of the four fixing bodies in the reaction; its direction can be reversed according to the respective importance of factors 1, 3 and 4.

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VIII. Nature and Mechanism of Anaphylaxis.

Knowledge of the nature of animal and plant tissues leads to knowledge of the nature of anaphylaxis. All anaphylactizing substances come from animal or plant organisms and are constituted by the organic mold *of which they contain the living reproductive elements.* If, when heated to 1000 or above, they lose this anaphylactic power, it is because, at this temperature, the reproductive elements of the organic mold they contain lose the power to reproduce.

Since the anaphylactizing substance is a mold, the state of *anaphylaxis is therefore a state of bacterial infection*, no different from infections of this kind. Any individual suffering from an illness caused by a heterogeneous germ therefore reaches the state of anaphylaxis.

From now on, in order to establish this point, I indicate that the simple demonstration is given by the following experiment: the guinea pig is injected with an anaphylactizing substance: horse serum, hen's egg albumin, bird serum, maceration of tissue from any animal, for example.

After fifteen or twenty days, if the guinea pig is not dead, it is killed and the pulp of its organs is cultivated on sugar agar; one or more gonidial forms of the organic mold of the animal that provided the injected antigen develop there; if, for example, parrot serum or turtle red blood cells have been injected, the organic mold of the parrot or that of the turtle will be obtained; if one wishes to convince oneself that this mold develops in the blood from the first days following the preparative injection, it can be obtained by culturing the blood by the methods indicated. It should be noted that the guinea pig is an animal extremely sensitive to these injections and often dies after ten to fifteen days; it is necessary to inject only a minimal quantity of substance.

This explains why it was possible (Roseneau and Anderson) to sensitize the guinea pig with infinitesimal quantities of preparative substance (1/100,000 cubic centimeter of horse serum). A very small quantity of vegetative elements of the antigen is sufficient to obtain the development of the culture in the organism.

The fact that anaphylaxis is a bacterial infection, like that caused by the inoculation of a culture, explains why *the injection of the serum or blood of the anaphylactized animal into another animal causes the same anaphylactic state in it, called, in this case, passive anaphylaxis.*

This designation is not exact, anaphylaxis being as active in the second animal as in the first, just as, when the disease of one animal is transmitted to another with its blood, it is as active in the second as in the first, except in the case where there is attenuation by successive passages.

We will now examine successively:

1. The development of the state of anaphylaxis.
2. The state of anaphylaxis or established sensitization.
3. Anaphylactic shock caused by a second injection of the preparing substance.

1. Development of the state of anaphylaxis.

What characterizes this period is the development of the bacterial culture of the mold contained in the preparing substance.

Proof of this development is done by culturing the blood on agar or by the other methods indicated. Among the molds obtained are one or more gonid forms of the organic mold of the antigen. We also obtain, in the form of semi-solid material, the bacterial culture characteristic of the antigen.

The bacterial culture of the antigen develops, in the blood, a considerable number of zymospores which constitute the proteolytic ferments whose presence Abderhalden discovered in the blood of the animals prepared. These zymospores have a proteolytic power that those of all molds possess, because they are the agents which develop and transform the nitrogenous materials necessary for the formation of basophilic protoplasm and hyaloplasma without which the mold cannot develop.

The primary injection of serum or any other antigen often causes, as we know:

1. Either an immediate attack of urticaria (injection of bovine or horse serum), the mechanism of which I have not studied.

2. Either early urticaria in the first three days following the injection, which probably corresponds to the development of the bacterial culture in the blood and the formation of colonies in the dermis.

3. Or late urticaria, from the eighth to the twelfth day; at this time, there are very large germinative masses of the antigen in vegetation in the blood, which, due to their size, must be stopped by the capillaries, where their development probably constitutes the urticaria papules.

2. State of anaphylaxis.

As is the almost constant rule for all molds, bacterial development, which takes place during an initial period of six to ten days (fermentative or bacterial phase), sometimes longer (typhoid fever), then passes into the mycelial state from the eighth to the twelfth day (mycelial phase). This state even begins to establish itself much earlier, because, if we examine the spleen of a guinea pig that has received a very pathogenic mold, tubercular for example, whose mycelium is easy to recognize, we find it already well developed on the fifth or sixth day. But it is preferable to consider the mycelial phase as established from the moment when, its development being very accentuated, the fermentative phase is, on the other hand, considerably reduced.

We know that this moment coincides with the establishment of the anaphylactic state, that is, the state that exposes the animal to anaphylactic shock if it receives a second injection of the antigen.

This state therefore corresponds to the existence in the blood, from this moment, of substances or elements which, by their new contact with the antigen injected a second time, cause the disturbance that constitutes anaphylactic shock.

3. Mechanism of Anaphylactic Shock.

To determine this mechanism, we must know the phenomena that accompany the shock. We already know that various important phenomena appear almost immediately after the determining injection:

1. The incoagulability of the blood.
2. The alteration of the complementary power of the serum.
3. A notable lowering of body temperature.
4. A notable decrease in blood pressure.
5. A decrease in the normal coefficient of intraorganic oxidations.

I sought to determine the determinism of these various phenomena; here is what I observed:

1. The notable lowering of temperature, linked to the decrease in the coefficient of intraorganic oxidations, results from a significant slowing of the blood flow rate. If we determine the quantity of blood flowing from a vein per minute, the jugular vein for example, we see that as soon as the shock is determined, this quantity becomes three to five times smaller. This phenomenon is very apparent to the eye, *the veins ceasing to be tense and collapsing as soon as the shock appears.*

The decrease in the coefficient of intra-organic oxidations is not due to a lack of oxygen in the arterial blood; this blood is bright, and its oxygen absorption capacity has remained normal; this fact had astonished Ch. Richet, who rightly observed **(1)** that the animal appears to be in a state of asphyxia even though the arterial blood is bright.

What matters for tissues, particularly for nerve centers and for muscles like the heart, is not primarily the proportion of oxygen contained in the blood, *but the quantity that passes into the tissue during the unit of time.* This quantity is a function of the coefficient of blood irrigation. In anaphylactic shock, this coefficient is reduced by three to five times, that is, three to five times less blood passes into a tissue than in the normal state.

The capacity of tissues to absorb oxygen is in no way affected, because the venous blood has become purplish and blackish and contains only a tiny amount of oxygen. The tissues have taken from the blood what they could; but, as the coefficient of blood irrigation has decreased by three to five times, *they were unable to find in the blood, during the unit of time, more than half of the oxygen they needed,* even by fixing almost all the oxygen in the arterial blood; hence, for the animal, an asphyxia that is not apparent, but real and effective, although the arterial blood is gleaming. In anaphylactic shock, death is due to asphyxia, the lack of oxygen coming from a considerable slowing down in the speed of circulation of the arterial blood.

2. The glycolytic power of arterial blood and plasma decreases in the blood after the shock. This fact is demonstrated by keeping in an incubator for six or twelve hours (24 to 48 hours for plasma) a sample of arterial blood or oxalated plasma, taken before the second injection, and another sample of arterial blood or incoagulable plasma taken after the shock and oxalated to equalize the conditions.

The glucose disappears more quickly in the first sample than in the second; the difference is irregular depending on the case; normal blood can destroy 30 centigrams of glycose, while blood collected after the shock destroys only 10 centigrams; in other cases, the difference is notably less.

This phenomenon does not exert a significant influence in anaphylactic shock, because the still significant proportion of glycose contained in venous blood (more than 50 centigrams per liter) shows that there was no insufficiency. But this phenomenon is nevertheless important to know because *it means that the agents that determine glycolysis in the blood have been affected by the phenomenon that causes shock.*

(1) *Ch. RICHET, Anaphylaxis.*

3. The incoagulability of the blood and the alteration of the complementary power of the serum are due to the same cause.

We have seen previously that the constituents of the fibrin-ferment or thrombin (cytozyme or lecithin, serozyme or zymospores) are also those of alexin or complement (terminal portion or lecithin, middle portion or zymospores) and that thrombin is confused with alexin; on the other hand, the glycolytic ferment is constituted by zymospores which it seems difficult to differentiate, morphologically, from those which constitute the serozyme and the middle portion of the complement.

The fact that the coagulability of the blood, the complementary power, and the glycolytic power of the blood are simultaneously altered proves that this alteration *results from that of an element presenting characteristics common to all three phenomena: the zymospores of the blood* (serozyme, the middle portion of the complement). These are obviously not destroyed, but placed in a state that prevents them from fulfilling their role, the hydrolysis of lecithins and fatty substances. We have seen previously that when this hydrolysis is prevented by deprivation of zymospores (or any other means), blood coagulation no longer takes place and the serum loses its complementary power.

Now, two phenomena prove to us that the zymospores are prevented from fulfilling their role because they are agglomerated in clusters and retained largely in the capillaries where they considerably slow down the speed of blood circulation; these two

phenomena are, firstly, the formation of clusters of granulations or myceles, observed in the blood and caused by the second injection of the antigen; secondly, the considerable slowing down of the speed of blood circulation. The obstruction of the capillaries is not in doubt, because here we see the presence of the bodies causing the obstruction, large clusters of granulations, and the effect produced, the slowing down of the blood flow.

The clusters of granulations in the blood are clusters of zymospores, because this liquid does not contain any other species of visible granulations.

There is therefore a close connection between all these phenomena which are in perfect concordance:

Blood incoagulability.
Alteration of complement.
Alteration of glycolytic power.
Formation of clusters of granulations in the blood, i.e., agglutination of zymospores.
Slowing of the blood circulation rate.
Insufficient oxygen absorption and asphyxia.

To fully understand the mechanism of anaphylactic shock, we still need to determine the cause that triggers anaphylactic shock, i.e., that causes the agglutination of zymospores.

Mechanism of sudden agglutination of zymospores caused by the second injection of antigen.

After the analysis made so far, it remains to be determined:

1. *What is the cause that determines the formation, in the blood, of clusters of formed elements that are the immediate cause of anaphylactic shock?*

2. Whether this agglomeration of elements results from a phenomenon of agglutination, coagulation, or precipitation?

3. Whether the normal elements of the blood of the prepared animal, those that determine its coagulation, and the active elements of the complement, in particular, actively participate in the phenomenon.

It appears, firstly, that the rapid agglomeration of elements causing shock cannot result from the phenomenon of specific precipitation, whatever its nature and considering it in its usual sense; indeed, it is a fairly slow phenomenon, which never occurs in two or three minutes like anaphylactic shock.

The rapid agglomeration of elements can therefore only result from a phenomenon of agglutination or coagulation. However, these two phenomena are confused, agglutination can only occur if the agglutinating substance is formed and this, which is fibrin, is formed by a phenomenon of coagulation.

The rapidity of appearance of the phenomena of anaphylactic shock indicates to us that the coagulation of the agglutinating matter (and the resulting agglutination of the formed elements) is a rapid phenomenon which occurs in two or three minutes.

To search for the causes and elements of this phenomenon of coagulation, followed by agglutination, we must refer to the following knowledge, already established:

1. The solution or emulsion of the antigen contains formed elements of its organic mold, with the hyaline substance and its formative elements.

2. The elements of the bacterial culture of the organic mold of the antigen (formed elements, hyaline substance, and its formative elements) exist in the blood of the prepared or sensitized animal.

3. The serum of the prepared animal acquires the agglutinating property for the formed elements of the antigen.

4. This serum also has the property of producing precipitation in the antigen solution.

5. Due to the specificity of the phenomenon, it is the elements of the same species that react with each other, that is, it is the elements of the bacterial culture of the organic mold of the antigen, contained in the blood of the prepared animal, that react with the elements of the antigen solution.

From this knowledge it follows:

1. That the blood of the prepared animal already contains, before the second injection, the agglutinating hyaline matter and its formative elements.

2. That the antigen introduced by the second injection contains a considerable quantity of zymospores which, when introduced into the blood of the prepared animal, can develop a phenomenon of rapid coagulation (in 2 or 3 minutes) of the elements forming the antigen hyaline matter, just as the zymospores of the blood leaving the vessels (serozyme) produce a rapid coagulation (in 2 or 3 minutes) of fibrinogen, the element forming the hyaline matter (fibrin) of the animal organic mold.

3. That the antigen introduced with the second injection contains a large number of formed elements (zymospores, spores, germinated spores having emitted a mycelial filament, etc.) which, introduced into the blood of the prepared animal, immediately fix the agglutinating matter it contains and can thus agglutinate immediately.

Thus, even without the formation of new hyaline matter following the second injection, the blood of the prepared animal already contains agglutinating matter or its elements and can quickly agglutinate the formed elements introduced with the second injection.

Experience also confirms this conclusion; in fact, an emulsion of antigen blood cells is agglutinated, often quickly, in a few minutes, by a minimal quantity of serum from the prepared or sensitized animal. It is obvious that this emulsion, injected into the blood of the prepared animal, will agglutinate almost instantly due to the enormous quantity of sensitizer (hyaline or agglutinating matter) in the presence of which it is found. Indeed, this emulsion, injected into an animal that has already received a preparative emulsion twelve or fifteen days previously, quite often causes anaphylactic shock, and even fatal shock. It is therefore the agglutination of red blood cells that, in this case, caused the anaphylactic shock **(1)**.

(1) *Intravenous injection.*

Now, any antigen contains figurative elements and is comparable to an emulsion of red blood cells These formed elements, introduced into the blood of the prepared animal, produce the same effect there: *the fixation and perhaps also the preliminary coagulation of the agglutinating hyaline matter of the antigen contained in the blood of the prepared animal.* These elements, loaded with agglutinating matter, clump together, *carrying with them the elements (zymospores or serozyme) of the organic mold of the animal with which they are intimately and regularly mixed in the blood,* and which, in the phenomenon, play only a passive role; but this entrainment, although only passive, has as a consequence the incoagulability of the blood and the disappearance of the complement by stopping the release of the acid portion of the lecithins (thrombin and alexin or complement, at the same time), the hydrolysis of these being stopped due to the agglutination of the zymospores which determine it and which constitute the ferment called serozyme.

Thus, complement and thrombin do not actively intervene in the production of anaphylactic shock; their disappearance is only a consequence of the immediate cause of shock, the agglutination into clusters of the formed elements of the antigen, as well as the fall in blood pressure, the reduction in the coefficient of blood irrigation and the resulting asphyxia, hypothermia, are only other consequences of the formation of these clusters and the obstruction of the capillaries which they cause.

* * * * *

CHAPTER VI

Through the studies presented in the preceding chapters, we have acquired new knowledge that will allow us to address the following subjects:

1. Does immunity exist and what does it consist of?
2. Serotherapeutic treatment in various diseases and preventive vaccination.
3. The treatment of human diseases by physical and chemical agents.

I. Does immunity exist and what does it consist of?

We have reached the end of the studies of the determinism of the principal phenomena on which the doctrine of immunity is based; it is now necessary to establish the significance of the observed facts. Let us first examine the successive stages by which this doctrine was established. This exposition is absolutely necessary to clearly establish the principles and the current conception of immunity.

It has been known for a century and a half, through Jenner, that the serosity of cowpox vesicles, inoculated into humans, protects them against smallpox. It has also been known for a very long time that, for certain diseases, an initial attack protects against a subsequent attack.

It was only in 1857, with Pasteur's memoir on lactic fermentation, that the era of determinism of the causes of diseases began.

Many studies quickly established that viruses are made up of figurative elements, the existence of which was noted as early as 1850 by Razer and Davaine in the blood of animals with anthrax, but without understanding their role.

This was followed fairly quickly by successive discoveries of the pathogenic agents of human and animal diseases.

From this moment on, the principle was accepted that the agents of diseases were bacteria, that is, microscopic agents taking the form of rods (bacilli) or granules (micrococci) reproducing directly, either by fission or by spores.

It was around 1880 that research on experimental immunization began to develop through Pasteur's work on *the attenuation of viruses*. Then, almost immediately, in 1882, Metchnikoff introduced phagocytosis, a normal physiological function that would destroy bacilli introduced into the body.

In 1888, Richet and Héricourt had the idea of injecting new animals with the serum of immunized individuals, and immediately afterward, Berhing and Kitasato (1890), putting this new notion into practice, discovered the antitoxic power of the serum of animals immunized against diphtheria and tetanus; then followed the work of Roux and his application to diphtheria.

The experiments of Bordet on the influence of immune serum on the cholera vibrio (1895), then of Erlich and Morgenroth, Bordet (1899), on specific hemolysis made it known that the immunizing antibody is a sensitizer, that is to say, a body which, by fixing itself on the antigen, sensitizes it to the dissolving action of the alexin of normal serum.

In 1901, Bordet and Gengou imagined the complement fixation or deviation reaction.

In 1902, Ch. Richet, then Arthus (1903), introduced anaphylaxis; since then, and despite a considerable amount of work, this phenomenon has not shed any light on the mechanism of immunity.

Subsequently, attempts at immunization multiplied, either by injections of live or heat-killed bacilli, or by repeated injections of toxin, or by injections of substances extracted from pathogenic agents.

Briefly summarizing these facts, we see that the principles of experimental immunity have been successively established:

1. By Pasteur's work on the attenuation of viruses (1880).

2. By the discovery of the bactericidal properties of serum (Büchner).

3. By the discovery of phagocytosis (Metchnikoff, 1883).

4. By serotherapy (Ch. Richet and Héricourt, 1888).

5. By the discovery of antitoxins (Berhing and Kitasato, 1890).

6. By the discovery of sensitizers or immunizing antibodies (Bordet, Ehrlich, and Morgenroth, 1895, 1899).

7. By the discovery of the complement fixation reaction (Bordet-Gengou, 1901).

Let us now summarize the studies conducted on the determinism of each of these phenomena and identify, from each of them, its significance with respect to the phenomenon of immunity, that is, with respect to the fact that a first attack of a disease, or the inoculation of a virulent or attenuated culture, protects against a second attack or inoculation or even only against the serious accidents of the first attack.

Jennerian Vaccination

Let us first determine the mechanism of the vaccinal state. The study procedures outlined so far make it easy to establish:

1. That the vaccine is constituted by a pathogenic mold, the organic mold of the potato.

2. That the inoculation of the vaccine confers the mycosis of this mold.

3. That this mycosis is permanent. I found the mold in the organs eight months after inoculation. I did not look for it beyond that, as all the rabbits I inoculated died two to eight months later. It is likely that it persists as long as the vaccination status.

Thus, we first note that if a rabbit to which we have inoculated the vaccine for more than twelve to fifteen days does not contract the disease again, it is because it exists in it, and the pathogen lives and vegetates in its organism in the mycelial form.

What then does the absence of a rash after the second inoculation mean? It obviously has a meaning identical to that of the result of a second subcutaneous inoculation of tuberculous culture (absence of cutaneous ulceration, or benign ulceration, Koch's phenomenon) or of a second inoculation of syphilis.

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A first inoculation of tuberculosis given to a guinea pig gradually causes cachexia over a period of fifteen to sixty days or more, and inevitably results in death. There is therefore no vaccination state conferred by the first injection; this is known and the fact is indisputable. However, the second injection causes a less serious skin ulceration than the first since it heals in a few days, while the first persists until death. The first injection therefore reduced the seriousness of the initial accident, but not that of the disease itself, the lesions of which are those caused by the mycelial vegetation of the pathogenic agent, a tubercular mold; in the same sense, one cannot think that a first inoculation of syphilis vaccinates because a second inoculation does not cause chancre; The individual who has contracted syphilis retains all its vitality, in his tissues, the living agent which is a mold.

A chancreous ulceration is *a small cutaneous abscess formed by the bacterial development of the pathogenic mold immediately after inoculation*; once the skin or mucous membrane is ulcerated and destroyed, *the mycelial growth which constitutes the indurated base of the ulceration begins, after a few days.* At the time of a second inoculation, and although the individual is not vaccinated against syphilis, but on the contrary contaminated, he is still, in fact, protected against the formation of a second cutaneous abscess followed by ulceration, that is, *against active bacterial development* of the newly inoculated pathogenic elements.

For the moment, we will limit ourselves to this factual observation: What constitutes the state of immunity against vaccinia is the development of the vaccine mold in the mycelial state in the protected organism.

This fact implies that, if the vaccine protects against the development of the acute period of smallpox, it confers on the organism a permanent mycosis, of which I was able to find some of the late accidents: ringworm (*Trichophytia*), herpes circinate and favus. Indeed, the pathogenic mold which is the agent of these accidents is the organic mold of the potato, agent of the vaccine and smallpox. Favus is a chronic form of the vaccine or smallpox eruption. These accidents clearly and materially establish that, if the inoculation of the vaccine protects against the acute phase of smallpox, it is because it confers the variola mycosis.

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Attenuation of viruses

A virus heated, or attenuated by any process, causes an attenuated disease, that is, one whose acute phase is benign; but, in fact, it causes the disease which, like any other, passes into the mycelial state in the organism for a certain period of time.

The organs of the animal inoculated with a culture of charcoal attenuated by heating reproduce the pathogenic mold by culturing the tissue pulp on agar. The vaccination state therefore differs from that created by the usual disease only by a less intensive development of the bacterial phase of the disease, a fact which results from the reduction of the vegetative activity of the pathogenic agent which constitutes the attenuation (Fact demonstrated by the study of ferments).

Once developed, the mycelium exerts its usual action in the chronic or mycelial phase.

Bactericidal power of the serum

It was Büchner who discovered this power, which he attributed to a substance he designated, alexin. To demonstrate this bactericidal power, Büchner inoculated a bacterial agent onto a gelatin plate and observed its development (number and size of colonies). Then he repeated the inoculation of the same culture by mixing it with varying quantities of serum, either normal or preheated to 55°C.

Under these conditions, he saw that normal serum prevents, at a certain dose, microbial colonies from forming and that this preventing power is destroyed when the serum is heated to 55°C. He also saw that a precipitating salt, sodium sulfate, added to the serum in a dose just sufficient to determine a clear start of precipitation, also causes it to lose its preventing or bactericidal power. Since then, this bactericidal power

has been accepted and still is. Let us analyze the significance of the facts discovered by Büchner.

Firstly, and practically speaking, his conclusion is inaccurate, since bacteria thrive in the blood of humans and animals, a fact attested to by the numerous bacterial diseases from which they are affected, particularly by epidemics of plague and cholera.

The conclusion is also inaccurate, *because the blood of animals is the liquid culture medium of their organic mold and contains bacteria, elements of this mold, in constant development.* It would be surprising if this medium, which combines the most favorable conditions for the development of bacterial or other elements of one species of mold, were unsuitable for the development of other species.

It therefore appears that Büchner's conclusion, only partially accurate with regard to serum, is not so with regard to blood.

Indeed, the properties of serum, *an immobile liquid* whose constitution gradually deteriorates, differ essentially from blood, *a circulating liquid with a constant constitution*, which possesses, in addition to serum, the elements it has lost through coagulation, notably fibrinogen.

It is easy to verify that, under *equal conditions,* more considerable hemolysis occurs in a mixture of red blood cells, serum, and sensitizer than in a mixture of whole blood and sensitizer. The restitution of fibrinogen to the serum does not restore complete equality, probably because blood coagulation and the precipitation of fibrinogen by a salt are not equivalent phenomena.

Apart from these considerations, the demonstration of the absence of bactericidal power in blood and even in serum is established by the following facts:

1. A small quantity, 0 cc. 05, or even less, of a 4 or 5 day-old culture of diphtheria bacillus, placed in a mixture of 1 cubic centimeter of fresh anti-diphtheria serum and 1 cubic centimeter of fresh guinea-pig serum (or 2 cubic centimeters of anti-diphtheria serum and 1 cubic centimeter of guinea-pig serum), then preserved in an incubator at 38°, evolves and multiplies there; a drop of this mixture, inoculated in broth after three days, reproduces the bacillary culture there with an evolution at least as rapid.

Thus, even in this case, where the complement fixation reaction is carried out on the bacterial elements, the culture vegetates in the serum and *suffers no damage there.*

2. The same reaction occurs in the blood of a typhus patient, where the typhus bacillus multiplies. This case can also be extended to that of the heterogeneous pathogenic agents of all other diseases.

3. The living figurative elements of the diphtheria and tetanus molds exist in the organism, in the blood, and in the serum of animals immunized by toxin injections; they are found alive in this serum after six months or a year.

4. Since pathogenic molds continue to vegetate in the organism, causing a long lasting mycosis, and since mycelial elements do not differ in any way from the bacterial elements which are their segmentation products, this fact demonstrates that blood has no bactericidal action.

If we add to these facts that the blood of an animal is the culture medium for its organic mold, and that, in fact, diseases caused by heterogeneous germs are the negation of a bactericidal power of blood, we must conclude not only that this liquid does not possess such a power, but that it constitutes a favorable culture medium for bacteria because of its richness in albuminoid matter.

If a certain number of bacteria vegetate less well in serum than in another culture medium, this fact in no way proves that it destroys them; it does not possess this power; one must conclude only that it is not favorable to the bacterial multiplication of certain species, that is to say, it only prevents, and nothing more, the multiplication of bacteria, but it does not kill them. Phagocytosis, lysins, opsonins... of leukocyte origin, do not exist.

Phagocytosis, lysins, opsonins... of leukocyte origin, do not exist

The phagocytosis described by Metchnikoff would be a normal physiological function of white blood cells, protecting the body against infectious germs. Immediately after the penetration of these germs, the white blood cells would be called to the site of infection and would phagocytose, that is, engulf and then digest the heterogeneous bacterial elements. In fact, in the white blood cells removed from a focus of infection, leukocytes containing bacilli are found, and the fact that the pus of an infectious abscess is composed mainly of an enormous mass of white blood cells seemed to confirm Metchnikoff's concept.

But how can we believe that phagocytes destroy infectious germs when the small quantity of germs introduced into the body by a bite is not destroyed? Why do they not prevent the infection from developing and often spreading widely through the lymphatic channels and nodes, which should be an elective site for the exercise of the phagocytic function?

In whooping cough, the trachea is covered with a mucous exudate containing a large mass of leukocytes that the patient expels to the outside. How can we believe that, to perform this phagocytic function, the leukocytes could exit the mucous membrane by diapedesis? This phenomenon is not at all established. On the other hand, it would be a singular physiological function that would fatally lead to the death of the organ that performs it; now the leukocyte that has crossed the mucous membrane is lost to the organism; the leukocytes of the pus would be doomed to death, an abscess opening naturally to the outside, in general.

How can we explain this fact, thus described by Bordet **(1)**: *"Do we not know that gonorrhea results from indigestion of the amoeboid cells, since the leukocytes that capture the gonococci do not succeed in destroying them and are, on the contrary, killed by the parasites that develop in them?"*

It must be recognized that there was a fatal error in phagocytosis that could only be avoided by knowledge of the nature of the animal organism.

It has been previously demonstrated:

1. That the bacterial culture of any mold, even in vitro, gives rise to agglomerated masses or germinative masses which are leukocytes.

2. That the introduction into the organism of the bacterial culture of a mold develops leukocytes that can be easily identified with those of the same culture developed in vitro.

3. That the animal organism is constituted by an organized mold which, like molds in vitro, forms germinative masses which are the normal leukocytes of the organism.

4. That, consequently, we encounter in the organism both normal leukocytes belonging to the organic mold and heterogeneous leukocytes formed by the infecting molds.

From these facts, a first explanation results: the leukocytes found in exudates, in the trachea, in the pus of an abscess, in gonorrhea pus, in cerebrospinal fluid that has become cloudy or purulent, *are not normal leukocytes; they are heterogeneous leukocytes formed by the infectious agent. They are the germinative masses of the latter.* If they are cultured, they give rise to a culture of the pathogenic agent.

(1) *Bordet, Immunity in Infectious Diseases, p. 161.*

The leukocytes in gonorrhea pus are agglomeration masses constituted by zymospores, spores, gonococci and bacilli; only elements of the pathogen enter into their constitution.

The observation of the development of bacterial cultures, combined with that of the formation of heterogeneous leukocytes in the organism, shows that the bacilli come to join the agglomeration masses like the granular elements and that it is basophilic elements (bacilli, spores, zymospores) which constitute the nucleus, while other non-basophilic elements (generally spores and zymospores) constitute the peripheral mass. The bacilli incorporated into the agglomeration mass appear *to dissolve there.* In reality, this is not a dissolution; it is only a fusion of the basophilic elements, resulting in the formation of a single basophilic mass (the nucleus) or of several masses which will later unite into a single one.

It is this phenomenon of agglomeration and fusion of bacilli or micrococci in the germinal masses (leukocytes) that has been taken for phagocytosis; the phenomenon, on the contrary, has an inverse meaning: it is the normal phenomenon of the formation of the leukocyte, and a stage in the evolution of the pathogenic agent.

A specific fact appeared to demonstrate in a decisive manner phagocytosis, that is to say the destruction of bacilli by leucocytes: Koch's bacillus is considered as a heterogeneous parasitic element; in the tubers, leucocytes containing a large number of Koch's bacilli are observed; it was logical to conclude that the bacilli were engulfed by the leucocytes. But, in this case, there was an inexact basis:

Koch's bacillus is not a heterogeneous parasite; it results from the segmentation of the fine mycelian branches of an abnormal, pathological vegetative form of the *organic mold of the subject;* on the other hand, the leucocyte is itself a mass of agglomeration of this pathological vegetative form, *because the presence of bacilli in its mass attests that it is in the process of formation*; There is even, between a human Koch bacillus and a normal human leukocyte, the specific link which explains the agglomeration of the bacilli with other normal elements to form a leukocyte.

On the other hand, even if it were a case of tuberculosis caused by a heterogeneous mold, to establish phagocytosis it would be necessary to demonstrate that the leukocyte which agglomerates the tuberculous bacilli belongs to the specific organic mold and is not a leukocyte of the heterogeneous tubercular mold.

The phenomenon observed in this case is therefore not phagocytosis, but the normal phenomenon of leukocyte formation.

The agglomeration of elements for the formation of leukocytes is a specific phenomenon; it occurs only between elements of the same species; it does not occur between elements of different species.

Elements derived from the organic mold of an animal can agglomerate with each other, but not with those of a heterogeneous mold. A human leukocyte cannot agglomerate a typhus bacillus, nor an anthrax bacillus, because they are specifically different. This fact explains the impossibility of a protective power of the body's normal leukocytes against a heterogeneous infection by bite, trauma, or ingestion.

Knowledge of the nature and mode of formation of normal or heterogeneous leukocytes, knowledge of the fact that *the engulfment of bacteria* by leukocytes is not phagocytosis, but the fundamental phenomenon of their formation, constitutes the demonstration of the non-existence of lysins, opsonins, or other protective antibodies that would be secreted by leukocytes, either to dissolve or to attract foreign germs.

Moreover, knowledge of the existence, in the animal organism, of leukocytes of two different natures: autogenous or normal leukocytes and heterogeneous leukocytes, knowledge of the rapid formation of the latter by the infecting agent, remove all meaning from the opsonic index and the leukocyte index; the number of normal leukocytes, increased by the appearance of heterogeneous leukocytes, is therefore in no way related to a defensive reaction of the organism.

It must therefore be concluded:

1. That phagocytosis is not a protective phenomenon against infectious germs that has the effect of destroying them; that it is, on the contrary, the fundamental phenomenon determining the formation of leukocytes.

2. That normal human leukocytes, the germinative masses of his organic mold, are incapable of agglomerating the bacterial elements of pathogenic and heterogeneous molds, which are specifically different.

3. That the variation in the number of blood leukocytes during an infection is related, not to a phenomenon of defense of the organism, but to the formation of heterogeneous leukocytes of the infecting mold and to their subsequent evolution.

Serotherapy.

Is there an immune reaction characterized by the formation, in the blood of a prepared animal, of an antitoxin neutralizing bacterial toxins?

This question has already been developed previously. It has been demonstrated, for example, that in a horse receiving diphtheria toxin, there cannot be an immunizing reaction in the body because:

1. The antitoxic serum does not kill the diphtheria agent; this agent develops and multiplies in vitro in the antidiphtheria serum preserved in contact with air and at a temperature of 18 to 22°C: multiplication takes place even in the presence of a quantity of serum 100 times greater than that of the inoculated culture.

2. The antitoxic serum does not prevent the toxin added to it from slowly transforming, in contact with air and at 18-22°C, into a bacterial culture; it therefore does not destroy the toxin any more than the culture.

3. The antitoxic serum contains the elements of the diphtheria mold; it can be extracted and cultivated even in a hyphomycete form.

These facts prove that there is no antitoxin in the anti-diphtheria serum, nor any body which, resulting from a reaction of the organism, would destroy the toxin or even prevent its development.

There is no immunizing reaction in the horse in the absolute sense of the word, since it actually contracts diphtheria.

But nevertheless, a substance is formed in the inoculated organism, the agglutinating matter, hyaline matter or mycofibrin of the diphtheria culture, which, mixed with the toxin, has the effect of accelerating its development and rapidly modifying its state. The zymospores of the toxin, which are its active element, quickly pass into the organism into the state of germinative masses. In this state, the fermentative action of the toxin is considerably diminished. It is to this modification of the morphological state of the toxin that the suppression of its tetanizing effect by the addition of antitetanus serum is linked.

But neither diphtheria nor tetanus toxin are destroyed by the serum, because the inoculation with the mixture of toxin and serum, or even the injection of the serum alone, kills the guinea pig after a certain time, and the tetanus or diphtheria molds can be removed from its organs.

Before going any further in this presentation, it is necessary to recall that a disease comprises two periods: the first is constituted by the bacterial development of the pathogenic mold in the organism: this is the bacterial or fermentative phase; the second is constituted by the period of mycelial vegetation of the pathogenic agent, a period which is always very long, lasting a very long time for certain diseases such as syphilis; for other diseases (malaria), the long mycelial phase is accompanied by a repetition, often frequent and periodic, of the bacterial phase.

The fact that the acute or bacterial phase ceases and its violent and rapidly evolving symptoms disappear does not mean that a disease is cured, as witness malaria, relapsing fever, late-onset accidents of many diseases, diphtheria, typhoid fever, scarlet fever, smallpox, measles, cerebrospinal meningitis, etc.

The second phase of the disease, much slower, often has more serious effects than the first.

It is therefore inaccurate to claim that a disease is benign because it does not have an acute period. It is no more accurate to believe that an immune reaction has been provoked because a subject has been given a disease that appears benign; the disease, passing into a lasting mycelial state, the subject is exposed to its effects and is therefore in no way immune. In horses immunized against diphtheria, there is development of the diphtheria mold. There is therefore no immunization in the exact sense of the word; there is only protection against the accidents of the acute or first phase of the disease.

By a first inoculation of vaccinia, the rabbit is completely immunized against the formation of new pustules by a second inoculation; but this is not an immunization, because the rabbit contracts the vaccinal mycosis, easily verified by organ culture, and always succumbs to this mycosis within two to eight months.

Can the antitoxic serum containing the elements of the diphtheria mold confer absolute immunity to a healthy subject? Obviously not, since it contains the pathogenic element capable of developing and, if it confers immunity against the acute phase, it confers at the same time the mycelial phase of diphtheria.

Is it by a real immune reaction that this serum acts on subjects already infected?

Two facts prove the contrary; first, the preservation of the vegetative power of the diphtheria bacillus in the anti-diphtheria serum; second, the absence of known protection of the anti-diphtheria serum against the late accidents of diphtheria, that is, against the accidents of the mycelial period.

The anti-diphtheria serum is therefore capable ***neither*** of stopping the evolution of the diphtheria mold nor of preventing it from developing in the mycelial state; its effect consists *only of a modification of the evolutionary form of the bacterial culture in the organism.*

It is therefore necessary to attribute to the anti-diphtheria serum not an immunizing action, but *only a restrictive action on the acute, bacterial period.*

From these facts, one must conclude:

1. That, in animals receiving repeated injections of a bacterial toxin, a body constituted by reproductive elements of the bacterial species and not soluble bodies, the pathogenic agent develops fully in the organism; they contract the disease caused by this agent.

2. That these injections do not provoke, in their organism, a defense reaction from which would result the formation of an immunizing body: an antitoxin. This cannot exist, since the multiplication of the diphtheria agent or the evolution of its toxin takes place even in a considerable dose of this serum.

3. That if the serum of these animals, injected into others, exerts an effective action against a simultaneous injection of toxin, it is by a modification of the evolution of the latter in the organism. Instead of evolving by reproducing zymospores over a certain number of generations, it passes more quickly to the bacterial, then mycelial, state.

4. That the body which exerts this modifying action on the evolution of the toxin in the organism is the agglutinating body which normally exists in any bacterial or mold culture; this body is manufactured by the mold itself or its elements, and no organic reaction intervenes in its formation, since it occurs in vitro, although to a lesser degree.

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Does the formation of sensitizers correspond to a defense reaction and a state of immunity of the organism?

It is necessary to first specify the conditions in which the infecting bacterial agents in the blood are placed with respect to the sensitizer developed by their culture in the organism.

It is accepted that a sensitizer is an immunizing body developed in the organism and whose fixation on the infecting agent facilitates its destruction by complement or alexin.

Now, complement is the animal's serum; it differs from plasma by the absence of fibrinogen. It was demonstrated above that the complementary property of serum disap-

pears if fibrinogen, which is a globulin, is restored to it, or if another globulin is added to it.

Alexin, consisting of the lecithin group (citozyme, terminal portion of complement) and zymospores (serozyme, middle portion of complement), does exist in circulating blood, *but it does not constitute the active body of complement,* the latter being the acid product of the hydrolysis carried out between the two constituents; on the other hand, this active body is fixed incessantly, as soon as it is formed in the blood, by fibrinogen, a body which is lacking in serum.

The complementary property of serum therefore does not exist in plasma, that is to say in circulating blood.

The absence of this complementary property in circulating blood demonstrates that it does not possess bactericidal power, which, according to the theory which led to its existence, depends on the interplay of two factors, the sensitizer and the complement, and cannot be exerted if one of these two factors is lacking.

If a hemolytic power has been observed in serum, it is because it is an abnormal liquid and the direction of the reactions in which it is the seat is distorted by the absence of the bodies removed by coagulation. We see, for example, that the complement or alexin, which is also thrombin, has the purpose not of destroying bacteria, but of coagulating fibrin, the formative element of organic mold whose mycelial filaments form bacteria by segmentation (See spontaneous coagulation of fibrinogen).

Under these conditions, since the complementary power does not exist in the blood, what use could the sensitizer have in the destruction of bacteria? It is obviously not formed for the purpose of this destruction.

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This conclusion is also supported by the following direct evidence:

1. By bringing bacteria, even hypersensitized ones, into contact with a very active supplement, there is no bacteriolysis; *nor is there the slightest bactericidal action, because if the bacteria subjected to this test is removed from the liquid after centrifugation and transferred to a culture medium, it vegetates and multiplies there.*

Thus, the complement fixation reaction, which undoubtedly demonstrates that the sensitizer binds to the antigen, in no way demonstrates that this fixation is detrimental to the latter. Verification of the vegetative and multiplying activity of the antigen proves, on the contrary, that it is in no way affected and *is, on the contrary, increased* in the elements which have bound both the sensitizer and the complement.

The only fact on which the existence of bacteriolysis has been established is Pfeiffer's experiment on the cholera vibrio: the transformation of the vibrio into granulations when it is transported in the peritoneum of an immunized animal (Pfeiffer) or in a mixture of cholera serum and complement (Bordet).

Bordet, who studied the phenomenon well, observed that, shortly after injecting vibrios into the peritoneum of an immunized animal, instead of the injected curved rods (vibrios), one finds only granulations or spherical micrococci; that sometimes, a vibrio, when rounded, provides a single granule; other times, it segments and produces two or three.

Very clearly, this phenomenon is not that of the bacteriolysis of vibrios, but the phenomenon, normal in any culture, of the segmentation of bacilli into micrococci, and the subsequent agglomeration of these into germinative masses.

If we subsequently find leukocytes in the peritoneal fluid that enclose the micrococci formed by the segmentation of the vibrios, this is not due to a phenomenon of phagocytosis, because these leukocytes *are not those of the organism, but those of the pathogenic mold*: they are formed by the vibrios themselves.

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There is therefore not a phenomenon of bacteriolysis, but, on the contrary, a phenomenon resulting in the proliferation, in different forms, of elements of the pathogenic mold of which the vibrio is only one element of the agglomeration masses; this phenomenon exists both in in vitro cultures of the vibrio and in most bacterial cultures; the bacillary elements most often fragment until the formation of granuations having their width as diameter.

The granulations thus formed are indeed evolving and not involutive, because, by searching in a preparation, we find clusters constituting germinative masses or leukocytes in the process of formation. It is enough to subsequently follow the evolution of these masses to realize that they are indeed germinative masses, because we always encounter some which have retained, still adherent, portions of mycelial filaments which they emit.

There is therefore no bacteriolysis in this case; there is an acceleration of a normal phenomenon of the evolution of vibrios.

2. In the blood of a typhus patient, bacteria continue to develop, although it contains the elements of the complement fixation reaction, that is, the elements necessary for bactericidal, sensitizing, and complement action. This fact is common to other diseases, as we know. We also know that the vitality of bacteria is in no way affected in the blood

of animals since, firstly, they abound there, and secondly, they actively vegetate in other in vitro culture media when they are transported there.

If Pfeiffer's experiment is carried out with Eberth's bacillus instead of the cholera vibrio, we see that it does not change shape and clumps together; however, this phenomenon is not bactericidal in nature; on the contrary, it is a phenomenon *that accelerates evolution*; it is therefore a phenomenon that increases the vitality of the pathogenic element.

The existence of a bactericidal power of the blood is, in short, a hypothesis suggested and based, mainly, on Büchner's first experiments on alexin and on an assimilation of bacteria to red blood cells, the latter being disorganized in a mixture of sensitizer and active complement.

It has been stated that Büchner's experiments in no way demonstrate the existence of a bactericidal power in the serum and even less in circulating blood.

As for the assimilation of bacteria to red blood cells, it is inaccurate, because, in the latter, two things exist:

1. the mold that constitutes them and which is capable of multiplying by forming bacteria;

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2. the organization of this mold into red blood cells. What specific hemolysis by a serum destroys is not the mold, it is only its organization. *If we examine under high magnification an emulsion liquid of red blood cells in the process of hemolysis or hemolyzed, we find a large number of constituent elements of the red blood cell in the process of vegetation.*

Let us now recall the results obtained previously in a study of sensitizers. The sensitizer is confused with agglutinin; it is the same body, of the fibrin type, which possesses both properties; the sensitizer, like fibrinogen and fibrin, possesses a strong affinity for the acid portion of the lecithin molecule; it is this affinity which determines the hemolysis of blood cells.

The fact that a sensitizer binds only to the element that determined its formation *proves that it is specifically bound to this element* and is formed by it and not by the organism. Agglutination occurs spontaneously in in vitro cultures as well as after the addition of agglutinating serum. Since the sensitizer cannot exert any action without the presence of complement, and since complement does not exist in the circulating blood, it follows that the hypothesis of an organic reaction, creating the sensitizer for the purpose of destroying bacteria, rests on an inaccurate basis.

It has been demonstrated above, in fact, that complement fixation on sensitized bacteria not only does not promote their destruction, but, on the contrary, accelerates their development. This is because the sensitizer is a substance formed by any bacterial culture and essential to its development. As in any bacterial culture, there normally exists, in any animal organism, a sensitizer that is also an agglutinin: it is blood fibrin.

What does immunity consist of?

We have just examined in succession all the facts on which the current explanation of immunity is based. We have seen:

1. That serum does not possess a bactericidal property, but only a preventing property, and that circulating blood possesses neither of these two properties.

2. That phagocytosis is not a defense phenomenon, but on the contrary a normal phenomenon in the evolution of bacteria and the phenomenon determining the formation of leukocytes.

3. That antitoxins are not bodies that destroy toxins in the true sense of the word; on the contrary, they accelerate their evolution by modifying their morphological state.

4. That sensitizers are neither lysines nor bactericidal bodies; on the contrary, they act on bacteria by accelerating their evolution.

A sensitizer is the fibrin from the bacterial culture of the antigen, and it is formed by the antigen itself. The simultaneous action of such an immunizing antibody and fresh complement on the antigen bacteria does not cause it the slightest harm.

When an infectious agent has invaded the body, none of the defense phenomena described so far intervene to expel it; in practice, the body does not destroy the infecting germ; when it disappears from the blood in its known form (bacillus or micrococcus), it is not by a reaction of the body; it is solely by the operation of its evolution; it changes form and passes into the mycelial state in the tissues.

At this moment, it is only the acute phase of the disease that is over; this persists and continues with the mycelial phase.

Immunity therefore does not exist in the absolute sense of the word for this disease, since it is always evolving.

However, indisputable facts prove that the first attack by certain pathogenic agents provides protection against a second attack. This is the case, for example:

1. For the inoculation of attenuated viruses such as those of chicken cholera, anthrax, etc.

2. For the inoculation of the smallpox vaccine.

3. After an initial attack by certain diseases and at least for a certain time (smallpox, chickenpox, scarlet fever, typhoid fever, etc.).

In these cases, since there is a proven protective phenomenon, what does it consist of?

To realize this, it was necessary to know that acute diseases, eruptive fevers, for example (smallpox, chicken pox, scarlet fever, measles, etc.), considered cured when the acute symptoms have disappeared, actually comprise two phases: one acute, short, and known, which is the bacterial phase; the other, chronic, long, unknown until now

for many diseases, *although its symptoms have not escaped clinicians; this is the mycelial phase, the late symptoms of which have been attributed to the persistent action of toxins impregnating the organs;* in reality, it is the mycelium of the pathogenic agent that invades them. The pathogenic agent persists in the body, constituting a mycosis, either transient or permanent like syphilis; it is to be believed, judging by the duration of vaccinal, smallpox, and syphilitic mycoses, that most of them are of very long duration.

Let us now examine the phenomena that accompany the inoculation of attenuated viruses or vaccinia, or infection by external pathogens.

In the case of inoculation of a virus attenuated by heat, for example, only a weak reaction occurs instead of the acute phenomena that follow inoculation of the virus possessing all its activity; but the phenomena determined by the injection are not limited to this observation; the virulent agent, affected in its vitality, *nonetheless develops in the organism and passes into the mycelial state.*

Two simple experiments make it easy to see the phenomena that develops in the injected organism.

Brewer's yeast and lab ferment, injected under the skin of the guinea pig's thigh, kill it in about ten to twenty days. If the injected product has been previously heated for 30 minutes at 58°, the animal dies later and presents a much lesser reaction after the injection.

It has been demonstrated elsewhere that ferments or enzymes are made up of elements formed from the organic mold of the animal or plant from which they originate, and that they have a practically inexhaustible activity because they vegetate and mul-

tiply. A ferment heated to 58° for thirty minutes loses its activity, as we know; *but this loss is not definitive.* The activity of the ferment reappears after a variable time (four to eight days or more), if it is cultivated in the nutritive medium that suits it; its vegetative activity, affected by heating, is gradually restored; brewer's yeast, which loses its power to transform sugar into alcohol when heated to 58°, recovers it after four to five days if it is cultivated on very sweet agar (5 to 10% glycose and 5 to 10% maltose); The smell of alcohol emanating from the agar tube easily provides information on the time of reappearance of the fermentative power.

Similarly, the lab-ferment heated for thirty minutes at 58°C loses its power to coagulate milk; if it is cultivated in a sterilized 5% lactose solution, its coagulating power reappears after three to four days.

It is the same in the body; the heated virus does not cause acute phenomena because, its vegetative activity being affected, its multiplication is slow; it thus reaches the mycelial phase without having developed sufficiently to cause the accentuated phenomena due to the proliferation of bacterial elements. It nevertheless reaches the mycelial phase which is established in all tissues, which is easily demonstrated by culturing the pulp of the animal's organs, lung, liver, spleen.

Inoculation of the heated virus therefore develops the same protection as the unattenuated virus, against subsequent infection, because it has developed the mycelial phase of the disease.

In the case of vaccinia, inoculation causes only a weak reaction, provided, as we know, that it is carried out in the dermis and not in the subcutaneous connective tissue. This process achieves the very slow dissemination of vaccinia zymospores and ensures the transition *to the mycelial state on site:* the slow development of zymospores throughout the organism, *up to the mycelial stage*, is ensured by the slow diffusion of zymospores. Here again, there is development of a long-lasting mycelial phase. After four to eight months, I found the vaccinal agent in the liver and spleen of the rabbit. This mycelial phase lasts, in humans, as long as the vaccinal state; it has been shown elsewhere that it is accompanied by accidents, some of which have been identified: trichophytosis, herpes circinate, and favus.

In the case of other vaccinating diseases, there is also the development of a mycelial phase, the existence of which is attested by well-known late disorders, which occur after the apparent cure (paralysis, liver disease, vascular disease, nephritis, etc).

These facts establish that, in all cases, the state of protection conferred by the disease *always coincides with the development of its chronic mycelial phase in the organism.*

It is not possible to oppose to this fact:

1. The state of protection created by an injection of anti-diphtheria serum as a preventive measure; this serum contains the elements of the diphtheria agent; its injection develops the diphtheria mycelial phase in the organism (experimental verification in guinea pigs by culturing their organs).

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2. The state of protection created by injections of toxin given to animals. The toxin is made up of reproductive elements of the pathogenic agent; its injection into the animal develops this agent in its entirety; it can be collected by tissue culture.

Anti-diphtheria serum from animals immunized with the toxin contains the diphtheria mold.

Thus, in all cases, there is a coincidence between the state of protection and the development of the mycelial phase of the disease determined by the pathogen.

We must therefore conclude that protection does not constitute immunity in the true sense of the word, since it can only exist simultaneously with the mycelial phase of the disease.

Let us now examine the phenomenon that achieves the protection conferred by the development of the mycelial phase of a disease, the process being the same in all cases, whether there has been a mild acute period (vaccination) or a severe acute period (contracted disease).

It has been stated previously that there is, in fact, no immunizing defense reaction that has the effect of impairing the vegetative activity of a pathogenic mold in the body.

The bodies that have been considered immunizing antibodies, sensitizers, or antitoxins, are, on the contrary, substances *produced by the pathogenic agents themselves and essential to their development;* a sensitizer, an antitoxin, or an agglutinin are one and the same very viscous, agglutinating body that energetically fixes organic acids (acid portion of lecithins and fatty acids); this body is a fibrin whose properties are identical to those of the fibrin produced by animal organic mold, an extremely viscous, agglutinating body that forms the blood or plasma clot.

It is this substance that causes agglutination. A culture passes all the more quickly to the mycelial stage as it forms germinative masses; it forms these masses more quickly if it is provided with the materials conducive to the formation of its fibrin.

However, a pathogenic mold that enters the body for the first time finds in the blood a favorable environment for its development. It produces its fibrin from the beginning of

its development, but only forms it in large quantities when it enters the stable mycelial period.

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The same mold, entering the organism a second time, finds its fibrin fully formed, developed by the elements already vegetating there. It therefore quickly forms germinative masses on the spot, that is, it quickly passes to the mycelial stage. It does not develop large quantities of zymospores in the blood, because these are agglomerated by the pre-existing fibrin.

Proof of the acceleration of bacterial evolution is provided by Pfeiffer's experiment (evolutionary acceleration of cholera vibrios by cholera immune serum). Proof of the rapid agglomeration of the elements is provided by the agglutinating action of the sera.

Finally, proof of the rapid transition of the culture to the mycelial state is provided by Koch's phenomenon; the second bacillary injection does not develop the same reaction as the first; the first injection causes, from the tenth to the fifteenth day, the formation of a hard nodule, the opening to the exterior of a small abscess and a persistent subsequent ulceration; the elements of the second inoculation *do not form a hard nodule and develop, after one to two days,* a purplish inflammatory zone which necrotizes in the following days, causing only a superficial ulceration which heals quickly.

Thus, the injected culture does not develop a mycelial nodule until the tenth to the fifteenth day after the first injection, while after the second, mycelial development has already begun after one day.

The particular significance of Koch's phenomenon lies not only in the rapid healing of the second ulceration, but above all in the almost immediate appearance of the ulcerative reaction after the second injection; the less seriousness of the second ulceration lies in the fact that the bacterial evolution of the injected elements is very rapid and that they pass almost immediately to the agglomeration stage and do not have time to multiply in large numbers before passing there, because they find the agglomerating matter on the spot and fully formed. Proof of the low multiplication of the elements after the injection and of their immediate passage to the mycelial state is provided by the ratio between the masses of indurated tissue (induration is strictly the zone of mycelial development) in the two cases; there are very few in the second case and the nodule is missing.

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Finally, another direct and decisive proof of the fact is constituted by the effect of the agglutinating or precipitating power of the serum of the sick or prepared animal on a culture of the antigen in vitro. *Microscopic examination of this culture, at various intervals, shows that there is an acceleration of its evolution.*

Thus there is not the slightest doubt to be preserved: *The infectious elements which penetrate a second time into an animal already carrying the infection in the mycelial form agglomerate on the spot and quickly pass there to the mycelial state before having been able to multiply notably in the bacterial form. The new mycelial vegetation has no harmful effect because the animal already possesses this vegetation extended to the whole organism;* the phenomenon thus takes place because the new infectious elements find on the pre-existing spot their hyaline matter (sensitizing and agglutinin) which causes their agglomeration.

Since the pre-existing hyaline or fibrin matter of the infectious element is produced by itself, the rapid agglomeration that constitutes the phenomenon of immunity or protection is therefore not a phenomenon of defense of the organism; it is a phenomenon that is only related to the evolution of the infectious agent.

This determinism entails important consequences:

The first is that, since the state of protection or immunity results from the establishment of the mycelial phase of the disease and cannot exist without it, *one cannot,* consequently, *immunize against the accidents of this phase.* This explains why the antidiphtheria serum does not protect against late accidents (those of the mycelial phase) of diphtheria.

On the other hand, it also follows from this fact that *it is impossible to immunize against diseases whose mycelial phase constitutes the only dangerous period,* such as syphilis, for example.

Nor is it possible to immunize against a disease which, in addition to a serious mycelial phase, presents spontaneous returns to the bacterial period, since the existing mycelial phase has not itself protected the patient against a new bacterial phase; malaria is the typical case.

Tuberculosis and cancer are serious diseases due to their mycelial period. For this reason, they are not amenable to an immunization process; *but they are not so especially because they are caused by a deviation of the vegetative form of the specific organic*

mold of the diseased organism, and there can be no question of immunizing an animal against its own organic mold or even against one of its abnormal vegetative forms.

Finally, there is a whole other series of diseases of humans and animals that have been attributed to the development of bacterial species and which also do not fall within the framework of vaccinal diseases, *because the bacterial species observed are formed by the organic mold itself. It has indeed been demonstrated elsewhere that, in a living being,*

animal or plant, organic mold can, if placed in unfavorable conditions, spontaneously transform into a bacterial culture.

This group of diseases includes pneumonia, pulmonary congestions, called idiopathic, certain diseases of the organs, liver, kidneys, nerve centers, vascular system, lesions of the organs characterized by the vegetation of the bacillus coli, a segmental form of the human organic mold, ulcerations of the mucous membranes presenting the fusiform-spirillus association, which are also segmental elements of the human organic mold, etc.

There are therefore only truly infectious diseases, with heterogeneous germs, where protection can be achieved, against the acute period only, by the development of the mycelial phase constituting vaccination.

* * *

The facts presented lead to the following conclusions:

1. Immunity does not exist; one cannot call immunity a state created by the development and persistence of the disease against which one wishes to vaccinate.

2. Vaccination confers the disease against which it is directed; the pathogen of this disease establishes itself in the organism in the mycelial form and causes a long lasting mycosis; the preservation of the vaccinated state is linked to the persistence of this mycosis.

3. Vaccination confers protection against the acute, bacterial phase of the disease, but not against the accidents of the mycelial phase since it develops them itself.

4. The protection achieved by vaccination at the cost of a long-lasting mycosis does not result from a phenomenon of defense of the organism; it results solely from the development of the mycelial phase of the disease and the presence, in the blood, humors,

and tissues, of the hyaline matter that the pathogenic mold itself produces for its development. This hyaline matter, or fibrin of the pathogenic agent, is the body that has been called sensitizer, agglutinin, precipitin.

5. Protection is effective during a second infection because the infecting bacterial elements, being in the presence of the fully formed hyaline matter, quickly pass to the mycelial stage; the bacterial phase is therefore short and poorly developed.

6. It is impossible to vaccinate against diseases whose serious phase is the mycelial phase: syphilis, malaria, cancer, tuberculosis.

7. It is impossible to vaccinate against diseases whose active agent is a vegetative form of the organic mold of the affected subject: cancer, tuberculosis, and all autogenous diseases.

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II. Serotherapy Treatment in Various Diseases and Preventive Vaccination

It is necessary to first recall the distinction that must be established between autogenous diseases of the animal's organic mold, against which serotherapy and vaccination appear to be powerless, and diseases caused by heterogeneous pathogenic agents.

Serotherapy and Vaccination in Diseases with a Heterogeneous Pathogenic Agent

From the preceding studies, it can be concluded, from the point of view of the curative treatment of diseases caused by heterogeneous germs:

1. That serotherapy treatment can only have an effect on the acute, bacterial phase of a disease, and that it acts by accelerating the development of the bacterial culture and its transition to the mycelial phase.

2. That serotherapy treatment, having no effect on the mycelial phase, is consequently ineffective in any disease consisting almost exclusively of a very long-lasting mycelial phase, such as syphilis.

It must be considered that, for diseases whose acute phase is generally benign, such as measles, chickenpox, scarlet fever, etc., it is not appropriate to use serotherapy treatment:

1. Because it does not protect against accidents of the mycelial phase.

2. Because a slight benefit would only be obtained in the acute phase of the disease at the cost of the development of the mycosis of the organic mold of the horse (or of another animal, depending on the origin of the serum).

For diseases whose acute phase is very dangerous, there is no doubt that, even at the cost of the horse's organic mold mycosis, it is appropriate to use curative serotherapy treatment; in the balance of risks incurred by the patient, the minimum corresponds to the application of serotherapy treatment.

On the other hand, in the mycelial phase of any disease, and particularly in diseases consisting of a serious and long mycelial phase, such as syphilis and malaria, serothe-

rapy treatment is contraindicated because, according to the facts presented so far, *it promotes the mycelial development.*

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In fact, the substance in the curative serum which exerts a favorable effect in the acute period, *by accelerating the transition to the mycelial phase, is the hyaline matter (sensi - tizer, agglutinin), the formative element of the mycelium of the pathogenic mold. It must therefore be avoided because it is impossible to prevent, by serotherapy treatment, the development of a pathogenic mold in the mycelial state in the animal organism.*

If the blood of a syphilitic human subject, inoculated into another, confers syphilis and not immunity, how could the serum of an inoculated animal contain curative or vaccinating elements that do not form in human blood? The treatment of syphilis by serotherapy is, obviously, powerless against the serious effects of this disease (secondary and tertiary effects of a mycelial nature) and *could only be aggravating.*

Preventive serotherapy

Preventive serotherapy exposes the injected subject to the two mycoses that the preventive serum can cause. The application of this procedure must therefore depend on the result of a balance between the risks or disadvantages of the disease against which one wishes to protect and the disadvantages of the mycoses that the subject is certain to contract. Preventive injection of the serum, done as a constant rule, is certainly a mistake if, keeping a subject under observation, one can begin curative treatment at the first sign of infection.

Preventive vaccination

Vaccination protects against the acute period of a disease and develops the mycelium of the pathogenic mold within the body; it follows that, if the severity of a disease is attached solely to the mycelial phase, vaccination is contraindicated because, instead of vaccinating, it will confer the disease in all its severity; this is the case, for example, with syphilis and malaria. The disease will be conferred even with a virus attenuated by heating, for example, because, as we saw previously, the vegetative activity of a virus, attenuated by heating to 57 or even 70°C, reappears after a certain time.

Vaccination is therefore only conceivable for diseases which, having a serious acute phase, have a less dangerous mycelial phase.

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If the inoculation of two different pathogenic germs, vaccinia and syphilis, appears to have such different effects, it is solely because of the different character of the two diseases, one, vaccinia, having an acute febrile period and a mycelial period which

generally appears to be of little seriousness, the other, syphilis, having a nil or insignificant acute period and having a serious mycelial period. In reality, the inoculation has an identical effect in both cases; only, in one, it confers an unnoticed mycelial phase because this is the character of the disease and, in the other, a serious mycelial phase. In both cases, the existence of the mycelial phase protects against a second development of the acute phase, that is to say, of the smallpox eruption or of the chancre with its initial acuity.

Due to the long-lasting mycosis that vaccination causes, this method is obviously not the best for protecting humans or animals.

The method of choice would consist of injecting a high dose of the specific agglutinating hyaline material of the pathogenic agent, completely devoid of reproductive elements. The injection of this hyaline material would not only be protective or immunizing for a certain time, but it would also have a powerful curative power because, due to its presence in the infected subject, the agglutination of the reproductive elements of the pathogenic agent would be achieved quickly, and the evolution of the bacterial phase would be notably shortened, *while the intensity of its acute phenomena would be lessened due to the agglutination of the zymospores.*

With this method, protection would only be short-lived, but could be renewed without inconvenience, since a product completely devoid of living, figurative elements would not be anaphylactizing.

Based on these facts, we can establish the following principle:

1. That vaccination, which necessarily involves the development of the mycelial phase of the disease in the vaccinated subject, should be carried out using an attenuated culture which only produces the necessary mycosis; a serum or other product removed from the animal also produces a second mycosis which is harmful and useless for vaccination.

2. *It is only possible to vaccinate against the bacterial or acute phase of diseases;* it is only appropriate to vaccinate against diseases with a dangerous acute phase and presenting fairly high risks of contagion, because of the defect that the vaccinating mycelial phase confers on the immune organism.

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3. *It is impossible to vaccinate against the mycelial phase of diseases since vaccination consists precisely of developing this phase;* it is therefore impossible to vaccinate against diseases whose severity is attached to the mycelial phase, such as syphilis and malaria. *In these diseases, vaccination will confer the disease itself.*

Serotherapy in autogenous diseases of the organic mold of humans and animals.

The organic mold of man appears in the organs and tissues in the organized mycelial form. Everywhere there exist, in innumerable quantities, very fine spore-like granules (0.2 to 0 μ. 8) animated by Brownian motion as soon as they are free: these are the zymospores of the mold, which also constitute the corpuscles designated mitochondria. In the blood, the lymph, there normally exist zymospores, spores and hyaline and basophilic bacterial elements in the process of incessant multiplication; the basophilic bacterial elements or the granules of 0 μ. 8 to 1 μ, to which they give rise, are the principal elements which, by their agglomeration with the germinative masses (leucocytes), provide the basophilic protoplasm which constitutes the nucleus.

Organic mold therefore normally forms bacterial elements, like any mold. When subjected to abnormal conditions, it can, at any point in the organism, transform into an abnormal bacterial culture.

This bacterial culture can transform into an abnormal mycelial form, different from the specific organic mold type, and whose autonomous vegetation will no longer be subject to the laws of organic development, although it has retained the original specific link with the organic mold. The treatment of organic mold diseases in animals cannot therefore fall within the rules established for the treatment of diseases caused by heterogeneous molds.

A disease characterized by the presence of a culture of a special bacterial form of organic mold, a coli form, for example, could not be safely treated with a serum from a horse injected with this bacillus or its toxin, because this serum could act in the body, not only on the coli-form bacillus, but *on other elements of the body that it risks agglutinating, causing a dangerous anaphylactic reaction.*

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The proof of this statement is contained in the fact that the bacterial culture, developed in the blood of an animal following an injection of blood cells, determines the formation of hyaline material of the antigen mold (sensitizer, agglutinin) which agglutinates fresh, normal blood cells of the same species.

In the treatment of autogenous diseases of human organic mold, it is therefore necessary to take into consideration:

1. The impossibility of preventing the mycelial development of a heterogeneous pathogenic mold in the body and, even more so, of causing its regression by serotherapy.

2. The possibility of promoting this mycelial development by injecting the serum of a so-called immunized animal; the antibody or sensitizer contained in this serum is the

mother substance of the mold's protoplasm (hyaline, sensitizing, and agglutinin substance); this serum also contains zymospores of the pathogenic mold.

3. The even greater impossibility of acting on the mycelial phase of a disease whose agent is a vegetative form of the specific organic mold; cancer and tuberculosis, for example.

4. The impossibility of acting on any other autogenous disease of the specific organic mold due to the specific link that exists between the abnormal elements (bacteria or mold) observed in the lesions and those of the normal organic mold.

These principles, progressively established in this work by the determinism of the various phenomena which had led to the belief in active reactions of defense or immunity in the organism, find their complete confirmation in the results of the trials of treatment of tuberculosis which will be found summarized and presented in the work of A. Calmette **(1)**.

Let us first note:

1. That human and bovine tubercle bacilli are respectively a bacillary segmentation element of an abnormal, pathological form of the organic mold of humans or cattle.

2. That if tuberculosis can be a disease contracted by infection, it is probably, in a large number of cases in humans, a *spontaneous* disease (pretuberculosis) resulting from the transformation of organic mold into an abnormal form under the influence of unfavorable causes.

It has been established by the research of numerous authors, whose results are consistent:

1. That antibodies are formed in the blood of tuberculosis patients.

2. That the level of antibodies, zero or low at the beginning of the infection, increases as the disease progresses.

3. That the antibodies disappear at the end when death is near.

From these facts, A. Calmette **(2)** concludes:

1. *That the development of antibodies by the tubercular organism always indicates a progressive form of the disease.*

(1) *A. CALMETTE, l'Infection bacillaire et la Tuberculose, 1920.*
(2) *A. CALMETTE, l'Infection bacillaire et la Tuberculose, p. 505-508, 1920.*

2. *That, in fact, repeated injections of tuberculin, live or dead bacilli, or their extracts aggravate tuberculous lesions and, despite this unfavorable action, increase the serum's antibody content.*

3. *That antibodies cannot therefore be considered the essential elements of defense against tuberculous infection and that they appear to be only the witnesses of cellular reactions against the toxic products excreted by the bacilli; that they do not neutralize them.*

The significance of these facts is as follows:

1. The proportion of antibodies increases in parallel with the amplitude of the vegetation of the tubercular mold.

2. The increase in the proportion of antibodies is a sign of aggravation of the disease because it indicates an increase in the vegetation of the tubercular mold.

3. The antibodies are not multiple; there is only one, the hyaline matter which is at the same time sensitizing, agglutinating and precipitating and which is the substance which forms the mycelial protoplasm of the tubercular mold; being elaborated by the zymospores of the latter and for its development, it is for this reason that its proportion is in relation only with the vegetation of the mold and not to a defensive reaction of the organism, the latter does not participate in its formation.

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We are authorized to conclude this because the nature and role of the hyaline material that constitutes the antibody (simultaneously sensitizing, agglutinin, and precipitin) have already been determined previously.

Can the antibodies contained in the blood of tuberculosis patients harm the development of the tuberculosis bacillus? We have already determined, by studying the action of the antibody or hyaline material contained in the anti-diphtheria serum, *that, far from harming the diphtheria bacillus, this substance actually promotes and accelerates its development.*

F. Meyer recommended, as a treatment for tuberculosis, injecting patients with progressively increasing doses of bacilli sensitized by their stay in an antituberculosis serum. In healthy animals treated with these sensitized bacilli, resistance to tuberculosis infection would be such that it would progress six to eight times slower than in controls. A. Calmette, checking this fact, saw, on the contrary, that healthy animals thus treated *often became tubercular more quickly and more severely than controls.*

The result obtained by A. Calmette is explained by the fact that sensitizing the bacilli consists of adding to them the hyaline substance (antibody contained in the serum),

which promotes and accelerates their development; this is therefore a condition for the aggravation of tuberculosis.

From these facts, it follows that:

Since the hyaline substance is the antibody that the tubercle-forming mold produces in an animal for its own development and since it only aggravates the disease, it seems obvious that the one developed in the serum of an animal that *one believes to be immunized* will have the same effect on the patient to whom this serum is injected.

Indeed, A. Calmette, studying the therapeutic action of serums containing antibodies, noted **(1)**: *"That sometimes those among them which are richest in antibodies hasten the progression of the disease instead of delaying it."*

Further on, A. Calmette concludes thus:

"By seeking to treat patients with injections of serum more or less rich in antibodies, agglutinins, precipitins, etc., one is probably making entirely vain efforts; *for, however*

rich may be, in these various substances, the best serums currently known, at the doses at which they are injected, they add almost nothing to the normally much more considerable quantities of antibodies, agglutinins, etc., contained in the mass of the blood of the patients themselves."

From this harmful action of antituberculosis serums, which should prohibit their use, one should not conclude that there is a contradiction with the real favorable effect of an antidiphtheria or antitetanus serum. *A therapeutic serum exerts a favorable effect on the patient during the acute phase of a disease because it shortens it by accelerating bacterial development; it exerts a harmful effect on the patient during the mycelial phase because it favors mycelial development.*

However,, diphtheria is a disease with an acute phase that is much more dangerous than the mycelial phase, while tuberculosis is more serious because of the mycelial phase that destroys the organs, than because of the multiple bacterial phases that it presents.

There is therefore no contradiction between the effect of an anti-diphtheria serum and that of an anti-tuberculosis serum; *in both cases, the serum acts exclusively on the development of the pathogenic mold and in an identical manner.*

***(1)** A. CALMETTE, Bacillary Infection and Tuberculosis, pp. 564 and 565.*

Impossibility of vaccination against autogenous diseases caused by an abnormal deviation of the specific organic mold.

Human organic mold can undergo multiple transformations that constitute autogenous diseases of the nervous centers, the lungs, the liver, the kidneys, the blood, nutritional diseases (diabetes, obesity, etc.), and above all the two most fearsome diseases: *tuberculosis and cancer. The transformation of organic mold into an abnormal mycelial vegetative form constitutes a mycosis.*

It therefore stands to reason that vaccination preventing organic mold from transforming into any abnormal mycelial vegetative form is not possible, since protection results precisely from the development of this mycosis; nor would it be possible to induce eight or ten vaccinating mycoses in a subject to protect him, since the specific organic mold can vegetate in at least eight to ten different gonidial forms.

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There are, however, cases of tuberculosis caused by the vegetation of a mold specifically different from the human tuberculosis mold, for example, bovine tuberculosis.

This distinction, which is important from the point of view of the etiology of the disease, has no consequences from the point of view of the possibility of vaccination. Since tuberculosis of bovine origin is a dangerous mycelial disease, it cannot be vaccinated against it.

What may have led to the belief in the possibility of vaccination against tuberculosis is the fact observed by Marfan**:** the resistance to tuberculous infection that appears to be possessed by subjects who have suffered, during childhood, from suppurative tuberculous adenitis of the neck and have recovered without other lesions; other lymph node lesions, childhood pleurisy, would also protect.

If this fact is correct, protection would result, according to what we have seen above, from the persistence of the mycelium of the living tubercular mold in the organism. It would result that the process of vaccination against tuberculosis would no longer be necessary; it would consist of infecting, in children, a distant lymph node with pus from tubercular adenitis.

Experimentation of this process is impossible in animals because the necessary conditions are unrealizable. Indeed, in humans, in the current case, there exists a specific link between their organic mold and the tubercular mold, and this link no longer exists in inoculated animals.

A vaccination experiment could probably only be successfully attempted in an animal by inoculation with the tubercular mold derived, by transformation, from its own organic mold, and only if it were carried out under conditions very different from those

which have been carried out until now. Indeed, it has been considered until now that tuberculosis can only be contracted by infection, which is obviously an error, since the bovine tuberculosis virus, being a vegetative form of the organic mold of beef, necessarily results from a spontaneous transformation of this mold.

It is therefore necessary to determine, first, the relative proportion of cases of spontaneous tuberculosis and cases of tuberculosis by infection, and then to demonstrate, in a vaccination experiment, that it prevents the spontaneous birth of the tubercular mold in the lung, in an animal placed in the conditions where this spontaneous development usually takes place.

We are naturally led to analyze the conditions under which Von Behring's bovine vaccination experiments were conducted and to identify their significance. Let us take as an example the experiment conducted by Vallée and Rossignol (Soc. de Méd. Vétérinaire, October 31, 1906). It should be noted in this experiment:

That Behring's bovine vaccination process used a culture of human tuberculosis, specifically different from bovine tuberculosis. It was therefore a heterogeneous germ that was inoculated into the animals, and it can be predicted, a priori, that germs of human origin should not develop substances that could reach germs of bovine origin.

Therefore, only a vaccine of bovine origin could vaccinate against bovine tuberculosis.

The test of the vaccinated animals was carried out with a virulent culture of bovine origin, that is to say, specifically different from the vaccinating culture which was of human origin. We have seen, up to now, that the only influence that the vaccinal state of the calves in the experiment could have had on the test culture was *the rapid agglomeration of the inoculated germs and not their destruction.* Now the vaccinating culture of human origin had developed in the subject's blood a hyaline matter which could not agglutinate the elements of the test culture, of bovine origin, that is to say, specifically different. It was therefore a bacillus of human origin which should have been used for this test. It follows from these observations, as well as from the preceding ones:

1. That a culture of human tuberculosis does not appear to be able to exert a vaccinating action against the contaminating elements of bovine tuberculosis;

2. That, even more so, vaccination with a culture of human tuberculosis cannot prevent the spontaneous transformation of the organic mold in beef into tubercular mold.

Let us recall that Vallée and Rossignol concluded from their experiments that:

1. The fairly clear resistance that vaccinated animals exhibit to venous infection, three months after bovine vaccination, is exhausted fairly quickly.

2. The resistance of vaccinated subjects to contagion, as it results from contact in the stable with animals carrying open tuberculosis lesions, is not very marked and does not last beyond a few months.

This experiment demonstrated, in short, that the animals were not vaccinated.

Furthermore, the test lacks an essential control element: that of the animals' resistance to the spontaneous development of tuberculosis.

For an anti-tuberculosis or anti-cancer vaccination to be admissible and effective, it will therefore be necessary to:

1. Induce the development of a strictly localized tuberculous mycosis with the tuberculosis virus derived from the organic mold of the vaccinated subject.

2. Be certain that this mycosis will not subsequently undergo a dangerous spread and will never become active, and that the virus will not undergo a transformation into other pathogenic forms.

3. Demonstrate that the vaccination protects not only against a test inoculation with a virulent culture, but also against the spontaneous transformation of the organic mold into tuberculosis mold.

These conditions seem unrealizable. Let us recall again:

1. That it appears impossible to vaccinate against tuberculosis because it is a disease with a dangerous mycelial phase and it is impossible to prevent the mycelial development of a mold, even if it is heterogeneous.

2. That it appears impossible to vaccinate against the vegetative forms of the mold that constitutes the organism, and that the tuberculous mold is one of these forms.

3. That it seems impossible to vaccinate against tuberculosis because it is often not a question of preventing infectious germs from evolving, but of preventing the spontaneous transformation of organic mold into tubercular mold.

* * *

III. Treatment of Human Diseases by Physical or Chemical Agents

Chemotherapy.

This method of treatment can yield some success in unfortunately quite rare cases; indeed, the nature of the animal organism makes the development of chemotherapy impossible and illusory for the following reasons:

1. Because the animal body is made up of a specific, organized mold.

2. Because, in organic mold, it is necessary to distinguish: the organization of the mold, and its vitality or resistance, considered in its unorganized or ancestral form.

The unorganized ancestral mold is very resistant to all agents and becomes pathogenic to the organism in this form; the organization is much less resistant than the mold that constitutes the organism. The loss of organization constitutes the death of animal or plant tissue; the tissue dies long before the ancestral mold is affected. In frozen tissue, for example, the tissues die and become gangrenous, but the vitality of the constituent or ancestral mold is not affected in the slightest.

3. Because unorganized heterogeneous molds generally appear to be as resistant as the unorganized ancestral mold of animals; they are more resistant to the action of physical and chemical agents than the organization of the specific mold constituting the animal body.

From these facts, it follows that a physical or chemical agent, acting on the animal body infected by a heterogeneous and pathogenic mold, will surely destroy the organization, that is, the animal, before reaching the pathogenic mold.

In syphilis, it appears that chemical agents exert a destructive or at least annoying action on the pathogenic mold; however, before drawing a definite conclusion on the effectiveness of old and new drugs used against this disease, it is important to verify whether, after their use, the syphilitic mold is destroyed.

Indeed, Schaudinn's spirilla is indeed a witness to the existence of syphilis, but is not its pathogenic agent; it is a mold whose mycelium is covered with spirilla: the ancestral mold of the monkey's organism (macaque). The disappearance of the spirilla therefore does not mean the cure of syphilis any more than the disappearance of Koch's bacillus means the cure of tuberculosis. What must be verified is the disappearance of the syphilitic mold itself. On the other hand, this mold vegetates in numerous gonidial forms

other than that which gives rise to spirilla, notably in the forms *Mucor, Penicilium, Aspergillus, Sterigmatocystis.* (See paragraph "Syphilis").

It is therefore also necessary to take into account the *possibility of transformation of the vegetative form into another that no longer produces spirillae* or that produces them of a different type (*Aspergillus* form).

In short, there is little to be expected from chemotherapy because, as a general rule, one cannot destroy an infective mold or even simply reduce its vegetative activity without at the same time destroying the organization of the specific mold, that is, the tissues, and without affecting the mold itself.

The same phenomena are found in the action of physical agents. It will be demonstrated later that cancer is caused by the vegetation of an abnormal form of organic mold; fundamentally, cancer does not differ essentially from tuberculosis. The decaying organic mold is diverted into its vegetative form; the new form has an evolution independent of that of the tissues, lives at their expense and causes them to disappear. The initial cause of cancer is therefore the cause of decay of the organic mold (trauma, prolonged inflammation, etc.).

Radiotherapy

The action of X-rays on radiograph tissues provides us with a demonstration of the action of physical agents. These rays exert a harmful effect on the organization of the specific mold, but not on the mold itself. Once the organization is affected, the organic mold tends to return to its ancestral type and vegetates in a new and independent form, which constitutes radiograph cancer. The destructive action of X-rays is quite intense, since a single application can develop the inflammatory phenomenon called radiodermatitis. Radium is known to exert a similar action.

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Given this destructive action on the organization of normal organic mold, but not on the mold itself, can we hope for an effective action of these agents in the treatment of cancer?

It seems impossible that one could obtain a result other than a vegetative modification of the cancerous mold having the effect of bringing it closer to the unorganized ancestral mold; this effect, which may appear to be an advantage while the therapeutic action is being exerted, is a disadvantage from the moment it ceases due to the new and inevitable evolution of the modified elements. In no case can one completely destroy or kill the cancerous mold either by a chemical or a physical agent; a vaccine or a therapeutic serum does not prevent it from evolving either.

CHAPTER VII - PLANT DISEASES

The study of these diseases requires knowledge of the multiple gonidial forms in which a mold of the same species can appear and, in particular, knowledge of the multiple forms that organic mold constituting a plant or an animal can take when it vegetates outside its organism. It is therefore with this study that this chapter will begin.

We will then proceed to study the following questions or illnesses:

1. Study of the symbiosis of plants with endophytic fungi and the relationships between this symbiosis and the tuberculation of plants.

2. Bacterial diseases of plants. Study of wet, bacterial rot of potatoes.

3. Study of potato diseases caused, according to current knowledge, by parasitic fungi, late blight, brown rot, yellow diseases, brown spot disease. Diseases of still unknown cause, crinkle, leaf roll, variegation, mottling, mosaic.

4. Study of fruit rot: apple and pear rot and its relationship with *Monilia fructigena*. Plum rot and its relationship with *Monilia cinerea*.

5. Study of vine diseases: *Plasmopara viticola, Botrytis cinerea*.

6. Study of lettuce sucker.

7. Study of parasitism of Uredinae: *Puccinia malvacearum; Puccinia glumarum; Puccinia tritici.*

8. Study of parasitism of Ustilaginea: *Ustilago aveni, Ustilago hordei, Tilletia tritici*.

9. Study of parasitism of *Nectria cinnabarina*, tree canker.

Multiplicity of gonidial forms of the same mold.

This question is of capital importance; if it had not been able to be resolved satisfactorily, most of the research that is the subject of this work would have remained sterile or its conclusions would have appeared tainted with inaccuracy.

How can we believe or make others believe that the same pathogenic germ can appear both in the form of a bacterial culture or a mold of the *Mucor, Aspergillus,* or *Penicilium* type, forms currently considered distinct genera?

How can we study a pathogenic germ if, injecting it in the bacillus form, we find it in the *Penicilium* form?

The plurality of gonidial forms of the same species is not a new concept; it was introduced into mycology by Tulasne, who showed, for example, that *I'saria farinosa* is a gonidial form of *Cordiceps militaris*. Brefeld, Istwanffy, and Johann Olsen showed that certain species of *Basidiomycetes* can give rise to gonidial forms reminiscent of *Botrytis* and *Polyactis*.

De Bary saw that the sclerotia of *Polyactis cinerea* can give rise to this gonidial form if sown on moist sand, or to *Peziza Fuckeliana* if buried one centimeter deep in the sand. Hamburg observed the transformation of the sclerotia of a *Botrytis* vegetating on rapeseed seeds into *Peziza cibarioïdes*.

We will see in this work that this plurality of gonidian or vegetative forms is much more extensive than we believed; it is very easy, for example, to obtain the transformation, into gonidian forms *Penicilium, Aspergillus, Monilia*, etc., of higher fungi, hymenomycetes for example, by cultivating on an appropriate nutritive medium, either their spores, or the product of aseptic scraping of the tissue of the foot or the cap, or by injecting this scraping product into the guinea pig and cultivating on agar the pulp of its organs.

This chapter is not concerned with the transformation of animal or plant tissues into molds, but with the passage from one type of mold to another, that is, with the determination of the relationships that exist between the various gonidian forms of molds, such as *Penicilium, Aspergillus, Sterigmatocystis, Acremonium, Verticilium, Sporothricum, Mucor*, forms that have been classified as distinct genera.

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We have seen previously that a bacterial culture can always be transformed into a mold having the form of an aerial hyphomycete. By carrying out this transformation, it happens that we obtain in one culture an *Aspergillus* and in another a *Penicilium*. Can the same culture give rise to two different gonidial forms? Can these two forms be transformed into one another, *Penicilium* into *Aspergillus* and vice versa?

From the beginning of my experiments in the direct in vitro transformation of bacterial cultures into hyphomycetes, I obtained, from a bacterial culture of anthrax (Bacillus anthracis), two different gonidial forms: a *Penicilium* form and an *Aspergillus* form.

I also obtained, with the cultures of Koch's bacillus, three gonidial forms: a *Penicilium* form, an *Aspergillus* form, and a *Sporothricum* form.

I previously explained how I was able, by the method of passing the cultures through the guinea pig's organism, to determine that these were multiple gonidial forms belonging to the same species.

These facts were verified by another method: the direct transformation, in vitro, of gonidial forms into other forms.

The microscopic study of certain cultures, particularly those that constitute the first strains of transformation of bacterial cultures into hyphomycetes and whose gonidial form is often not yet fixed in a characterized type, allowed me to observe the stages of the passage from one gonidial form to another.

These observations allowed me to establish that *the various gonidial forms are neither genera nor species, but only stages in the evolution of the same hyphomycete culture, and that each gonidial form represents only a stage of arrest or the provisional fixation of this evolution.*

The evidence for the multiplicity of gonidial forms of the same species of mold is therefore provided by three types of research or observations:

1. By the transformation of one gonidial form into another in vitro.

2. By the transformation of one gonidial form into one or more others by inoculation into the guinea pig and culture of the animal's organs (lung, liver, spleen, lymph nodes).

3. By microscopic observation of the cultures.

1. Direct transformation of one gonidial form into another in vitro.

A whole series of demonstrations relating to this subject has already been given in another chapter; it concerns the transformation of the bacterial vegetative form of molds into a vegetative form of aerial hyphomycete, affecting either the *Penicilium* type, or the *Aspergillus* or *Sporothricum* type, etc.

By directly cultivating gonidial forms of aerial molds in vitro, I was able to obtain the following transformations:

A. *Direct transformation of the yeast form (Saccharomyces) into the Sporothricum form and of the latter into Penicilium.*
Transformation of the Oidium form into the Aspergillus type.

I obtained the first transformation using a culture of brewer's yeast on agar from Mr. Sabouraut's laboratory and using brewer's yeast made available to me by Mr. Jenné, director of the Sochaux brewery in Montbéliard.

Brewer's yeast, cultivated in beef broth with peptone and glycose at 1 part per 1000, stored at room temperature, that is, around 18 to 20°C, initially produced a cloudy culture broth, then slowly formed a veil after about two weeks. This veil, transferred to carrots, gave rise after four or five days to a hairy culture of hyphomycete, a *Sporothricum*; one of the cultures of this *Sporothricum* spontaneously gave rise, after about a month, to a colony of *Penicilium* (Fig. 1 to 6, pl. 306), which is a gonidian form of organic barley mold.

I also obtained the direct transformation of brewer's yeast into this same *Penicilium*, without passing through the *Sporothricum* state, by inoculating a culture broth of brewer's yeast from Mr. Sabouraut's laboratory onto sterile droppings.

Here, there can be no doubt about the connection between the *Saccharomyces, Sporothricum, and Penicilium forms, because the culture of this Penicilium on sugar agar (10% maltose, 10% glucose) has retained the fermentative properties of brewer's yeast and transforms the sugar into alcohol.* The quantity of alcohol formed in the culture is so notable that one can perceive its very strong odor without removing the cotton plug from the culture tube.

Moreover, I obtained another confirmation of the accuracy of this transformation by the method of passing the yeast into the guinea pig's organism; I thus obtained *the same Penicilium* form, possessing the same fermentative properties as above, and, in addition, the *Aspergillus* form of the yeast by culturing the lung, liver, and spleen of the guinea pig. The culture of this *Aspergillus form on sugar agar does not transform the sugar into alcohol.*

Here we find the explanation of a fact well known to brewers: the disturbance that occurs in the fermentation of beer when a new culture appears in the vats, which they call yellow sarcina. This is the transformation of brewer's yeast into its *Aspergillus* form, the culture of which becomes dark yellow and which no longer has the property of transforming sugar into alcohol; this transformation seems to take place especially when the temperature of the vats rises above the optimum temperature.

I also transformed a yeast commonly used by bakers to make bread dough rise into *Penicilium*. This yeast, spread on very sweet agar, gave me a mold of the *Penicilium* form. The pure culture of this *Penicilium* on sweet agar gives off a strong alcoholic odor, and, in addition, an odor reminiscent of that of bread fresh from the oven. The same *Penicilium* form was obtained by passing the yeast through the organism of the guinea pig and culturing the latter's organs on agar.

I performed the direct transformation of a culture of lily of the valley (*Oidium albicans*) from Doctor Sabouraut's laboratory by culturing it on egg yolk; it gave rise to an aerial mold of the *Aspergillus* type.

We can conclude from these results:

That the genus *Saccharomyces*, as well as the neighboring genera (*Oidium*), and the species they include, are based on characters without specific or generic value, *the yeast form being only a stage of development, in liquid or semi-liquid media, of species of molds vegetating in several aerial gonidial forms, notably in the Penicilium and Aspergillus forms.*

B. *Transformation of the Mucor form into the Penicilium form.*

I obtained this transformation in cultures of *Mucor* developing on the pulp of brain, liver, spleen, etc., cultivated on agar, of animals inoculated with certain pathogenic germs.

The first strains of certain species have a remarkable tendency to pass to the *Penicilium* type and do indeed pass there, giving rise, in the middle of the *Mucor* vegetation, to one or two green colonies which spread rapidly over the entire surface of the tube.

This tendency is such that, whatever precautions one takes to inoculate only the terminal fruiting bodies of *Mucor*, and even after several successive transplants, the transformation still occurs; a colony of *Penicilium* often appears as early as the fourth day of culture.

I have obtained this rapid transformation in several species, notably in the *Mucor* form of a cholera vibrio (Bologna vibrio) and in the *Mucor* form of the pathogenic agent of typhus. Subsequently, I observed the fact in other molds (*Botrytis cinerea, Bremia lactuca*), etc.

I will not insist here on demonstrating that the *Penicilium* obtained is indeed a gonidial transformation of *Mucor*, because I was able to catch the birth of *Penicilium* in the act, that is to say, the first stage of the transformation. The transition to the *Penicilium* form occurs through the birth, on the *Mucor* mycelium itself, of short gonidial stems of *Penicilium*, bearing sterigmates emitting the first spores that will be the origin of the first colony of *Penicilium.* We can thus find, on the same mycelial branch, a typical *Mucor* fruiting body and a gonidial stem of *Penicilium* (Fig. 567, pl. 228).

Figures 6, 7, plate 150 show this transformation on the mycelium of *Botrytis cinerea* of the vine.

C. *Direct transformation of the Penicilium form into the Mucor or Rhizopus form.*

This transformation is rarer than the previous one. The *Penicilium* form of the horse's organic mold gave rise to the *Mucor* form after three subcultures of the *Penicilium* culture.

I was provided by the cryptogamy laboratory of the Museum with a pink *Penicilium* (*Penicilium roseum - Patouillard*) collected from a palm tree (*Howea Forsteriana*) in the greenhouses of the Museum. The spores of this *Penicilium*, inoculated on very sugary agar, give rise to a pink colony which, when it has reached a certain extension, transforms around its circumference into a black *Rhizopus*. Once I obtained directly and immediately the development of this *Rhizopus*.

Pink *Penicilium* reproduces in the same form while retaining its color. However, it is only one stage in the transformation of a mold; this pink *Penicilium*, inoculated into a guinea pig, allows one to obtain its green *Penicilium* form, whose microscopic appearance is very different.

By inoculating the guinea pig with palm leaf pulp (taken from the midrib), I obtained a green *Penicilium* identical to the previous one. This fact indicates that the pink *Penicilium* found on certain necrotic parts of *Horvea Forsteriana* is a gonidial transformation of the organic mold specific to this palm.

D. *Direct transformation of the Monosterigmata (1) form into the Penicilium form.*

This mode of transformation is not rare. I have obtained it on several species of mold. I cite here only the most remarkable transformation. The *Monilia* form of the pathogen of this disease can be found in the plates accompanying the article Syphilis.

This characteristic *Monosterigmata* form spontaneously gave rise, on the periphery of a culture about three months old, to a colony of the *Penicilium* form of syphilis. The following paragraph, E, also explains the justification for the transition from the *Monosterigmata* form to the *Penicilium* form by the fact that the *Monosterigmata* form can itself develop on the mycelium of the *Mucor* form.

E. *Transition from the Mucor form to both the Monosterigmata and Penicilium forms simultaneously.*

I observed this transition on the *Mucor* form of the pathogen of typhus. The mycelial branches of this *Mucor* produced thin lateral branches bearing the characteristic

(1) *I bring together in this form different types which are difficult to distinguish from each other and which have been designated until now Monilia, Torula, Acremoniella.*

fruiting bodies of the genus *Penicilium* and other very long, flexuous branches bearing isolated appendages in the form of sterigmata (*Monosterig- mata* form, fig. 5, 6, 7, 10, 11, pl. 228).

F. *Transition from the Aspergillus form to the Sterigmatocystis form and vice versa.*

Mr. Mangin, professor of Cryptogamy at the Museum, gave me a culture of *Aspergillus repens* dating from 1892. The spores of this culture, although thirty years old, began to vegetate eight days after being inoculated and reproduced *Aspergillus repens.* This culture, replanted the following year, gave rise to a *Sterigmatocystis*.

I isolated, for the first time, the rabies agent in the Aspergillus form shown (Fig. 1, pl. 278). This *Aspergillus*, pricked onto agar six months later, evolved in a different form, the *Sterigmatocystis* form.

I isolated from the cerebrospinal fluid of a patient suffering from a condition diagnosed as encephalomyelitis, which was in reality a form of encephalitis lethargica, a characteristic *Aspergillus* that I identified as *Aspergillus minimus* (Bainier) (Pl. 246). This culture, subcultured several months later, developed in the form of *Sterigmatocystis*.

The culture of this *Sterigmatocystis,* carefully examined under the microscope, contained all the forms of passage between *Aspergillus minimus* and *Sterigmatocystis*, which leaves no doubt that *Aspergillus minimus is an incompletely developed form of Sterigmatocystis.*

Finally, the culture of vaccine pulp on agar develops a *Sterigmatocystis* form of vaccinia that is difficult to preserve in its entirety, in its pure form, because it always transforms at certain points into the *Penicilium* form.

G. *Direct transformation of the Sporothricum form into the Penicilium form.*

I obtained the progressive transformation of a culture of *Sporothricum Schenk*i that I had from Doctor Gougerot, a physician at the Hospitals. In a first stage of transformation, I first succeeded in obtaining on carrot, then on agar, a very hairy, white culture. In a second stage and after five or six subcultures, the culture evolved into a rudimentary white *Penicilium* type. This latter type evolved spontaneously, on the periphery of a two-month-old colony, into its perfect *Penicilium* form, the culture of which is green (Fig. 1, 2, pl. 281).

I also obtained the transformation of a culture of *Sporothricum Beurmani*, which I also had from Doctor Gougerot. I first succeeded in obtaining a hairy culture, in a veil on broth, of this *Sporothricum.*

This veil, transferred to carrot, gave me a hairy culture with long hairs, 3 to 4 millimeters long, dark gray, which spontaneously transformed, at the periphery of a 3 month-old colony, into a green culture of *Penicilium* (Fig. 4 to 8, pl. 290).

I isolated, from a cancerous tumor of the uterus, a *Sporothricum* form which is a gonidial form of the organic mold of man. The culture of this *Sporothricum* form (Fig. 1, pl. 189) is hairy and creamy white in color.

There are fruiting bodies typical of the *Sporothricum* form and a whole series of fruiting body forms evolving towards the *Penicilium* form (Fig. 2, 3, pl. 189). There are, on the other hand, other fruiting bodies which have retained a more rudimentary form and are identical to forms found in connective tissue or other organic tissues.

One of the agar cultures, about three months old, spontaneously transformed into the *Penicilium* form (the culture of which is green) *of the organic mold* of man (Fig. 4 to 9, pl. 189).

One might assume that these transformations of gonidial forms are not real and are the result of contamination introduced into the cultures during transplanting. A fairly rapid experiment demonstrates that this assumption is erroneous. Injecting the original culture into a guinea pig makes it possible to obtain, by culturing the lung, liver, and spleen, the gonidial form identical to that resulting from direct in vitro transformation.

H. *Relationships between the Fusarium, Sporothricum, and Penicilium forms.*

By culturing whiting organs (Fig. 8, pl. 323), I obtained a mold whose fruiting bodies are of the *Fusarium* type and which spontaneously passed to the *Penicilium* type (Fig. 1 to 7, pl. 323). The same fact was reproduced with turtle tissues.

The cultivation of sterile potato pulp produces a *Fusarium* (*Fusarium solani*), which is the vegetative form of the potato's organic mold, a fact demonstrated by a series of research studies presented in this work.

I directly obtained the transformation of a culture of *Fusarium solani* into *Penicilium*. I noted not only the modification of the culture that, from white, becomes green, but also the stages of transformation.

The mycelial filaments of *Fusarium solani* are, in pure and typical cultures, long, sparsely branched and covered with characteristic fruiting bodies. When the mold passes to the *Penicilium* type, the number of *Fusarium* fruiting bodies decreases more and more and the mycelial filaments are covered with lateral branches ending in a tapered end rich in basophilic protoplasm. These lateral branches emit small round spores identical to those of *Penicilium*. Later, we see that these ramifications, as well as the ends of the branches, develop, at their ends, the groups of sterigmata characteristic of the *Penicilium* form. It is also noted that the mycelial filaments bearing the fruiting bodies typical of the *Penicilium* form have retained the compartmentalization and the typical, easily recognizable appearance of the *Fusarium* mycelial filaments. It is also observed that, in these same bundles of mycelial filaments, some still bear the fruiting bodies characteristic of *Fusarium*, while others bear *Penicilium* fruiting bodies.

These different stages of transformation will be found photographed in Plate 148 and described in detail on the subject of the diseases called dry rot and wet rot of the potato (page 346).

The *Fusarium* form of organic mold in corn always transforms spontaneously into the *Penicilium* form: the branches of the latter are born in large numbers on the mycelial filaments of *Fusarium*.

I. *Relationship between the Verticilium form and the Penicilium form.*

Culturing the dog's brain matter pulp on sugar agar gave rise to both the *Penicilium* form of the animal's organic mold and a *Verticilium* form.

Culturing the pulp of the organs of an eight-day-old young caiman (*Alligator mississipiensis*) gave rise to a *Penicilium* form and a *Verticilium* form. When the latter was injected into the guinea pig, culturing the animal's organs on agar gave rise to a *Penicilium* form identical to that obtained directly from the caiman's organs. We will see elsewhere that the organic mold of the potato transforms, in vitro, into the forms *Fusarium, Verticilium, Spicaria, Penicilium*, etc.

K. *Transformation of the vegetative or gonidial forms Trichophyton, Microsporum, Achorion.*

I have obtained the transformation of these three genera of molds into the gonidial forms *Monosterigmata* and *Penicilium*; I will not expose here the study of these transformations which is done elsewhere. I will only point out that it has been determined

that the most common *Trichophyton* and *Achorion* are diverse forms of the same mold: the organic mold of the potato, agent of cowpox and smallpox.

2. Transformation of one gonidial form into one or more others by the passage of cultures into the animal organism and cultivation of its crushed organs.

A bacterial culture or a mold culture being inoculated into an animal, its organs (lungs, liver, spleen, lymph nodes, brain) reduced to pulp and spread on agar can give rise to:

A. One or more gonidial forms of its specific organic mold.

B. Several gonidial forms of the injected mold.

Almost always, it is the injected mold that grows first and, by the third day, generally produces aerial colonies, often very numerous, particularly on the pulp of the lung. *This organ is a preferred location for the vegetation of foreign molds introduced into the organism.*

I used the organism of the guinea pig and sometimes that of the rabbit for my transformations. Photographs of the *Penicilium* form of the organic mold specific to these animals can be found in plates 282, 312, 313, 314. Since these figures are known, it is possible to avoid confusion between the pathogenic molds studied and the organic molds specific to the inoculated animals.

I will cite only two examples here to demonstrate what use can be made of this process to establish the link between the various gonidian forms.

A culture of lily of the valley spontaneously gave rise to the form *Rhizopus nigricans*. Culturing the organs of two inoculated guinea pigs, one with the lily of the valley culture, the other with *Rhizopus nigricans*, gave rise in both cases to the form *Penicilium.*

By culturing the lymph removed from a syphilitic chancre and extremely rich in spirillae, I obtained a mold of the *Monosterigmata* type (Fig. 1, 2, pl. 283). By culturing the pulp adhering to the crust of this same chancre, I obtained another mold of the *Mucor corymbifer* type. I was unaware at the time that a connection could exist between the two types. Six months later, a four-month-old culture of the *Monosterigmata* form spontaneously evolved at its periphery to the *Penicilium* type (Fig. 1 to 8, pl. 285).

The culture of the *Monosterigmata* type, injected into the guinea pig for control purposes, gave rise, by culture of the animal's organs:

1. To the *Monosterigmata* form identical to the injected form.

2. To a mold of the *Sterigmatocystis* type

3. A mold of the *Aspergillus minimus* type.

4. A mold of the *Aspergillus glaucus* type.

The *Penicilium* form did not exist in the cultures. To determine if these different gonidial forms are related to each other, the *Sterigmatocystis* form was injected into a guinea pig. The culture of this animal's organs gave rise to:

1. A *Penicilium* form identical to that obtained by spontaneous evolution of the *Monilia* form.

2. The same *Aspergillus minimus* form obtained in the first guinea pig. Fruiting bodies of this *Aspergillus minimus* form were also observed in the culture of the previous *Penicilium*, and even on mycelial filaments bearing *Penicilium* gonidiophores.

3. A *Mucor corymbifer* form.

These facts therefore establish that the five gonidian forms: *Monosterigmate, Sterigmatocystis, Aspergillus, Penicilium, Mucor corymbifer*, belong to a single species of mold.

3. Microscopic observation of hyphomycete cultures.

By careful observation of cultures, we can observe a series of transition forms between gonidial forms that are quite distant from each other, the *Monosterigmata, Aspergillus*, and *Penicilium* forms, for example.

We can even, by systematic research, observe on the same mycelial branch the presence of fruiting bodies characteristic of two very different gonidial types, for example, gonidial fruiting bodies of the Penicilium type on the mycelial filaments of a *Mucor.*

We have already seen previously that the same mycelial branch can bear at its ends fruiting bodies of the *Monosterigmata* type and, at other points, fruiting bodies of the *Penicilium* type. This is a very common occurrence. For example, in the *Sterigmatocystis* type, we frequently encounter, following the ramifications of a mycelial branch:

1. The typical, fully developed fruiting bodies of *Sterigmatocystis.*

2. Long, less bushy gonidial stems, now presenting only a limited number of sterigmata and which exactly represent the form of an *Penicilium aspergilloïde.*

3. Lateral branches bearing four or five appendages in an umbel, each ending in a group of sterigmata. These branches have the shape of an *aspergilloid Penicilium*.

4. Short branches bearing a single group of four or five sterigmata at their ends.

5. Isolated sterigmata on the branches and representing the *Monosterigmata* type.

6. Long gonidial stems identical to those of the perfect *Sterigmatocystis* type and presenting the *Aspergillus* form; this results both from the coalescence of the branches bearing the groups of sterigmata and from the insufficiency of development or the more or less accentuated coalescence of these.

It is enough to examine a certain number of cultures of *Sterigmatocystis* relating to dif - ferent species to observe all the forms I have just indicated. Several examples will be found in this work, in plates 187 and 248, for example.
Here are various examples which establish, by direct observation, the transition from one gonidial form to another.

1. The culture of the pulp of various organs of a young caiman (*Alligator mississipiensis*, eight days old) gave rise to a culture of the *Sterigmatocystis* type (Fig. 4, 5, 6, pl. 320) presenting fructifications of the *Penicilium* type (A, fig. 5, pl. 320). In this last figure, we notice a gonidial stalk B, of the Aspergillus type and of a frequent form in cultures of this type, the end of which bears the index of the division manifested in the perfect state in the fructifications of the *Penicilium* type.

2. I obtained, by culture of the serosity of the pustules of a patient suffering from chickenpox, a colony of the *Aspergillus* type which presented fruiting forms so remarkable that I photographed them and reproduced them in plate 140. In figs. 1 and 2 of this plate, we will see gonidial stems of the usual form in *Aspergillus.* In fig. 3, we also notice fruiting heads of a common form, but two to three in number at the end of branched gonidial stems. In figure 1, we see that certain sterigmata have evolved in the form of filaments emanating from the fruiting head.

In Fig. 4, we see a bifurcated gonidial stem bearing, at the end of each of its two branches, a group of very long sterigmata emitting strings of spores. Fig. 5 shows a stem divided into four branches bearing fruiting bodies of a common form. The most curious and significant forms are represented in Figs. 6, 7, and 8; examination of the fruiting forms in Figs. 2, 6, 7, and 8 represents, beyond any doubt, the evolutionary tendency of the mold from the *Aspergillus* type (Fig. 2) to the *Penicilium aspergilloid* type (Fig. 8).

4. On another mold representing the *Mucor* form of typhus exanthematous, I made even more convincing observations demonstrating, beyond any possible doubt, that when a mold that has passed into the bacterial state resumes its hyphomycete form, it can develop under the most diverse gonidial types, for example, under the *Mucor*, *Monosterigmata*, and *Penicilium* types.

I was struck by the fact that, however meticulous the precautions taken to inoculate only the gonidial ends of this *Mucor,* one always sees a *Penicilium* developing on the surface of the agar, after the development of the *Mucor.*

Investigating the cause of this fact by microscopic examination of the culture, I saw, developed on the mycelial filaments bearing fruiting heads of the *Mucor* type, gonidial stems characteristic of the *Penicilium* type (Fig. 5, 6, 7, pl. 228).

Continuing the study of the culture, I also noted the existence of fine lateral branches emanating from the *Mucor* mycelium and bearing the characteristic fruiting bodies of the *Monilia* type (Fig. 10, 11, pl. 228).

The microscopic study of mold cultures has provided me with a large number of observations of this kind. There are very few species that I have studied without them providing me with observations supporting the facts set out above.

The reader who studies the numerous photographs contained in this work will also be able to find other examples similar to those I have cited so far. To list them all here would be too long and, moreover, useless, the demonstrations that I have given being sufficient to provide clear, decisive proof of the accuracy of the facts of capital importance that follow.

* * *

The results provided by the various methods of observation or experimentation used lead us to formulate the following conclusions:

1. *Any mold of a given form can be transformed into numerous other gonidial forms by appropriate means; this transformation occurs frequently and spontaneously in in vitro mold cultures.*

2. *The specific mold constituting any living organism, animal or plant, can develop in multiple gonidial types: Mucor, Monosterigmata, Sporothricum, Aspergillus, Sterigmatocystis, Penicilium, Fusarium, Verticilium, etc.*

3. *No gonidial form is stable and definitive; it can always be transformed into another form.*

4. *The molds called Mucor, Sporothricum, Monilia, Sterigmatocystis, Aspergillus, Penici - lium, Verticilium, etc., cannot therefore be classified as either genera or species. They are only gonidial types: the species is characterized by the series of all its gonidial types and by the forms it takes in each of them.*

5. *Within the same gonidial type, the fruiting form is not rigorous; it presents variations establishing its affinities with the closest gonidial types.*

Characterization of gonidial types

Any botanist who has extensively studied the morphology of molds is certainly convinced that one of the most accurate classifications that can be applied to them is that of *Adelomycetes* (Mangin). Indeed, under the influence of variations in the composition of culture media, temperature, and humidity, they can exhibit variations that make them unrecognizable. The transformations just studied are proof of this.

Currently, hundreds of genera, that is, gonidial types, have been described. Are they legitimate? It is enough to examine certain categories of these genera to answer in the negative.

For example, the genera *Uromyces, Phragmidium, Fusocladium*, and *Pleurophragmium* have been created to designate different forms which are, obviously, only varieties of the same gonidial type which can be called *Fusophragmium*. These genera are therefore not legitimate.

The genus *Monilia* is not legitimate, since its typical fruiting bodies are found on mycelial filaments that also bear fruiting bodies of *Aspergillus, Sterigmatocystis,* or *Penicilium*. The genera *Torula* and *Acremonium* are no more legitimate: the plates in this work contain a large number of undescribed molds, some of which could be assigned to all three of these genera at once, so poorly characterized are they.

When we examine molds of the *Monilia fructigena* type growing on rotting fruit and compare these forms to others included in the same genus, we are struck by the fact that they could have been grouped together. On rotting plants, a mold identical to *Monilia fructigena* often develops, but which has been designated by the name *Cladosporium*; it is clear that here, it is the habitat of the mold and not its morphological characteristics that have caused it to be distinguished and assigned to a special genus. One of these two genera was therefore useless.

I could multiply these examples for most of the genera or species created, because, from the examination I have undertaken, it appears that there are hardly more than twenty gonidian types that deserve to be retained.

Moreover, it is demonstrated later in this work:

1. That Uredinaea and Ustilaginea do not exist. Rusts and smuts are a vegetative aspect that organic mold takes on when plants suffer.

2. That Peronosporae are an external vegetative form of organic mold in plants.

3. That Ascomycetes, numerous Basidiomycetes, Oomycetes, and bacteria are vegetative forms taken by diseased organic mold.

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If some of these forms cannot be reproduced in cultures, it is because they can only exist as organic mold, that is, in connection with the plant environment; as soon as they are transported to artificial environments, they lose the type they affect on the organism that gives rise to them and develop in a gonidial form of hyphomycetes.

A striking example, which reproduces for most plant organisms, shows how little value the characters on which the current classification of fungi is based have.

Organic mold in wheat can give rise to:

1. A species of Peronosporiacea *(Sclerospora graminicola)*.
2. Two species of Ustilaginea *(Ustilago tritici, Tilletia tritici)*.
3. Two species of Uredinaea *(Puccinia graminis, Puccinia Glumarum)*.
4. To several species of Spheriaceae *(Ophiolobus graminis*, for example).
5. To the entire series of gonidian forms of Hyphomycetes, *Penicillium, Aspergillus, Monosterigmata, Fusarium, Fusophragmium.*

Organic mold in wheat can therefore, depending on the evolution it undergoes, appear in the form of an Oomycete fungus *(Sclerospora),* Basidiomycetes *(Ustilago, Tilletia, Puccinia),* Ascomycetes *(Ophiolobus), or* Hyphomycetes (numerous gonidian forms).

Organic mold specific to vines can evolve in the form of:

1. An Oomycete *(Botrytis cinerea*, which is confused with *Plasmopara viticola*).

2. An Ascomycete *(Guignardia Bildvelli)*. In addition, *Botrytis cinerea*, an Oomycete, can transform into a Peziza *(Peziza Fuckeliana)*, which is an Ascomycete.

3. Numerous gonidian forms of Hyphomycetes.

For these two plants, the same mold, the one that constitutes the plant organism, can evolve rapidly, under the influence of various conditions, and become indifferently an Oomycete fungus, a Basidiomycete, an Ascomycete, or a Hyphomycete.

How, under these conditions, could we consider as genera and even as species forms that do not possess the criterion of the latter: the stability of characteristics?

From the studies presented in this work, it emerges:

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1. That organized mold is stable because it reproduces with fixed, constant, and always similar elements.

2. That unorganized mold (which always comes from a living organism, animal or plant) has no stability because it can reproduce with any element that constitutes it, whether with a fragment of mycelium, an intra- or extra-mycelium spore, or a gonidia.

It therefore follows from these facts that fungi cannot be classified and distinguished as is done in the current classification. When they are not clearly organized, like the higher fungi, they can only be taken for gonidian or vegetative forms of the organic mold of the animals or plants from which they come.

They must therefore be given a designation comprising the name of the plant or animal species from which they come and the name of the gonidian type they affect. It is obvious, from what has been explained so far, that *Guignardia, Pseudopeziza, Sphaerella, Hypoderma, Nectria,* etc., are identical deformations of organic molds of plants and that they differ from each other only because the plants that carry them are different. They must therefore be given a general designation.

On the other hand, the name *Nectria cinnabarina,* for example, has been confused with a pathogenic formation that develops on a large number of plants and which, despite its apparent identity, differs for each of them; in fact, that of each plant, cultivated outside of it, gives rise to different hyphomycetes in the same gonidian form. We must therefore distinguish the *Nectria* by adding the name of the plant and call the *Nectria* of the plum tree, for example, *Nectria Pruni*.

Many species of *Penicilium* and *Aspergillus*, currently described, each contain a series of different species, but with forms so similar that they cannot be distingushed from each other, for example, *Aspergillus glaucus*. They are species because they come from different animal or plant organisms; they can only be distinguished by knowledge of the living being to which they belong and by comparing all their gonidial forms as well as their fermentative properties.

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For the study of the determination or origin of species and genera, it is necessary to take as a basis for comparison a certain number of reliable, common gonidial types that reproduce easily, such as the *Mucor, Monosterigmata, Sporothricum, Fusarium, Verticilium, Sterigmatocystis, Aspergillus, Penicilium*, etc. types.

A large number of gonidian types have been described as genera, the conservation of which is impossible because they are in the process of evolution; they have no legitimate existence because they are only generally rudimentary, often accidental, states of the defined gonidian types.

To avoid confusion in the nomenclature of the gonidian types discussed in this work, the main ones have been grouped in their most common form in plate 141. Each of

these types presents a certain number of fixed forms which will be described and shown elsewhere, but which are contained in this work, scattered throughout the plates.

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Nature of Plant Diseases

We will only discuss here diseases whose cause is attributed to parasitic cryptogams, bacteria, or fungi. The majority of plant diseases fall into this category.

These diseases are considered infectious and contagious, mainly because it has been accepted in principle that a cryptogamic or bacterial species observed on a plant is a foreign and infective parasitic agent.

However, it has been found that many species of parasites that practically only exist on a specific plant species affected by the disease attributed to this parasite cannot reproduce the disease by inoculation on other individuals of the same species.

It has even been found that the bacteria that exist in the lesions of certain plants, for example, those of bacterial wet rot of potato tubers, do not reproduce the disease when inoculated into healthy tubers.

In reality, the exact cause of these plant diseases has remained unknown until now. It remained unknown because the observers who studied these parasites were unaware of the intimate constitution of plants and the possibility of gonidial transformations of the organic mold that constitutes them.

Cryptogams or bacteria, considered as causes of diseases, are not parasites of foreign origin to the plant and introduced by infection. *They are parasites created from scratch, spontaneously, in the plant*; they arise from the organic mold that constitutes it and are only transformations of it; this abandons the normal vegetative type that it possesses in plant tissues and transforms into an accidental or pathological gonidian form, when it is subjected to certain unfavorable conditions in the environment in which it lives.

These facts are not specific to plants; they also apply to a category of animal diseases whose cause has been attributed to parasites observed in tissue lesions (autogenous diseases of the organic mold of animals).

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Here are the facts that explain the genesis of the diseases covered in this study:

1. When the specific mold constituting living organisms is subjected, locally or in its entirety, to unfavorable conditions: too high or too low a temperature, insufficient or excess humidity, nutrients, fertilizers in insufficient or excess quantities, introduction

of toxic substances, etc., it can lose its normal vegetative type and transform into other vegetative (bacterial or mycelial) or gonidial forms.

2. The new vegetative form born from the normal specific organic mold no longer obeys the laws of its development and develops in a different way; its chemical nutritional reactions, its growth rate, its mode of multiplication are different; its fermentative properties, the substances it produces are no longer the same.

In summary, the new vegetative form lives exactly like a parasite at the expense of the still healthy tissues with which it is in contact.

It is therefore not correct to consider bacteria as always being *agents of infection of external origin* to the organism in question. In animals, it is often the specific organic mold that gives rise to them spontaneously within the organism itself; in plants, this fact seems to be the constant rule.

Here are some examples of these facts for clarification:

A. The organic mold that constitutes an apple transforms (normally on fruit that has fallen to the ground) into *Monilia fructigena*, and the sclerotia of this form give rise to a peziza *(ascomycete fungus: Stromatinia fructigena*). The apple does not rot due to infection by *Monilia fructigena*; it rots because this is its normal physiological evolution; *Monilia fructigena* is a gonidial form of the elements of normal fruit rot.

B. Under the influence of cold and soil moisture, potato tubers are affected by a so-called bacterial rot (wet rot). The bacteria that exist in this rot result from the transformation, in a bacterial culture, of the organic mold specific to the potato.

C. Phytophtora infestans, considered the cause of the disease called potato late blight, is a gonidial form of the normal, specific organic mold that constitutes tubers.

Phytophtora can be reconstituted in vitro by culturing the normal organic mold of the potato taken from *the aerial stem of healthy specimens.*

D. Downy mildew and powdery mildew of the grapevine are gonidial forms of the normal organic mold of the grapevine developed under the influence of a condition unfavorable to it: excess moisture.

* * *

The facts being specified and materially fixed by these examples, let us examine the real causes of diseases. Let us take the case of wet rot of the potato occurring during vegetation. We know that it only occurs when the earth remains excessively wet for too long due to continuous or too frequent rains. This excess of humidity is the cause of the mortification of the organic mold in the tubers; the organization disappears and the

mold undergoes a bacterial evolution. Here, the presence of bacteria is therefore not the cause, but only the witness of the mortification. The real cause is the excess of humidity and the lowering of the temperature: the bacteria is only a consequence of this cause, although, later, its development can be the cause, real this time, of the destruction of the entire tuber, if the primitive causes have disappeared.

Similarly, if *Phytophtora infestans* and downy mildew appear in tubers or on the surface of leaves, it is because a change in humidity and temperature conditions modifies the vegetative form of the organic mold; these two molds are therefore only manifestations and consequences of the initial cause of the disease and not the cause itself.

Indeed, it is very easy to develop the *Peronospora* form of any plant (in the form *Botrytis cinerea*). Simply place the leaves of this plant in a stoppered crystallizing dish containing a little sterile water and placed at a temperature of 24-25°C. *Botrytis* cinerea is generally seen developing after two to four days. The cause of the development is obviously excess humidity, *combined with excessive heat*, be- cause leaves placed in an unclosed crystallizing dish remain free of *Botrytis.*

This demonstration is completed by in vitro transformation (which has been operated on) from the healthy organic mold of the potato tuber into its gonidial form *Phytophtora infestans* (which is a form of *Peronospora* or *Botrytis cinerea*); the latter is therefore not of exogenous origin and cannot, consequently, be the initial cause of the disease; it can only be the one that determines the transformation.

Thus, in summary, diseases are or can be spontaneous in plants; their immediate causes are unfavorable modifications of the external environment in which the plant lives; these modifications lead to those of the internal environment and those of the specific organic mold; the latter gives rise to an abnormal gonidial form which, from the moment it is formed, lives in the plant as a parasite.

Cannot this, in turn, infect a neighboring healthy plant of the same species and even a plant of a different species?

The fact is perhaps possible; I believe that the tests of contamination of cereals by rusts are conclusive; on the other hand, direct observation of natural infection provides proof of it.

By examining on a tree two apples or two pears which are in direct contact and one of which is rotten, we see that the rot passes to the healthy fruit exactly through the point of contact and that it spreads, forming a circle whose center is the point of contact.

Natural infection therefore exists. It is obvious that it is all the easier when the subjects it affects are already tested by the unfavorable conditions which caused the spontane-

ous disease in their neighbors. It would be very important to know, for each disease, the share which is due on the one hand to spontaneous outbreak and on the other to contamination. The distinction may be possible in certain cases with careful and continuous observation of the individuals.

For example, in the case of bacterial wet rot of potato tubers, we generally note that only a certain number of tubers are affected in each plant; a good number remain unaffected. In this case, there is therefore no contamination, because, if it existed, all the tubers would be affected in the same plant, due to their almost immediate proximity. We also know that, experimentally, this contamination does not succeed, even by inoculation.

The fact that rot can be transmitted from one tuber to another or from one fruit to another when they are in a silo, cellar, or storage does not allow us to conclude that the same contamination occurs in standing plants; the conditions are very different in the two cases. For example, rotten tubers or fruits exude a liquid which, by the water alone it contains, is enough to rot the individuals it touches.

Growers know that tubers (and other vegetables in general) or fruits rot easily if they are brought in wet from the rain and grouped together.

I will not dwell further on this question of contamination in plants. My goal here was simply to point out:

1. That the demonstration of the spontaneous outbreak of a disease in an individual does not exclude the possibility of contamination of neighboring individuals by this same disease.

2. That if contamination exists for certain diseases, it has been attributed an importance that it does not have, these diseases developing spontaneously in a large number of individuals.

What has just been explained does not result from more or less plausible hypotheses; these are only the consequences of facts whose precise demonstration will be given, progressively, by the successive study of the diseases listed at the beginning of the chapter.

* * *

Study of the symbiosis of plants with endophytic fungi and the relationship of this symbiosis or infection with plant tuberculation.

I recall, at the beginning of the study of this question:

1. That plants, like all living beings, are made up of an organized mold.

2. That plant tissues, aseptically detached and suitably cultivated, give rise in vitro to multiple gonidial forms of the specific organic mold.

A large number of molds considered to be parasites of fungi (mainly *Basidiomycetes*) have been described because they were observed developing on them and, most often, exclusively on a specific species. These molds were not parasites, but a gonidial form of the organic mold specific to the fungus. By culturing tissues, taken aseptically, from the middle of the stem and the cap, these gonidial forms of the organic mold are obtained; molds of the types *Mucor, Sporothricum, Monosterigmata, Acremonium, Verticilium, Citromyces, Penicilium, Aspergillus*, etc. are obtained.

We should therefore not conclude that a mold is parasitic because it is found developing on a plant.

It was Franck's **(1)** work that highlighted the presence of endophytic fungi parasitizing higher plants; *he considered this parasitism to be a symbiosis*, with infected individuals suffering in no way from the presence of the fungus. Wahrlich **(2)** showed, on more than 500 orchids, that the presence of endophytic fungi is constant in this family.

Schlicht **(3)** observed the same fact on a large number of herbaceous plants. Janse **(4)** recognized that, in a tropical forest, 69 out of 75 plants, belonging to various families, were parasitized. Stahl **(5)** then cites new cases of parasitism.

We are informed by the facts demonstrated so far about the significance of the presence of these endophytic fungi observed by Franck, Wahrlicht, Schlicht, etc., in the normal and healthy tissues of plants. These fungi are gonidial forms of the specific organic mold constituting plants.

(1) *B. FRANCK, The Rooting of Orchids on the Symbiosis of Javanese Plants (Report of the German Botanic Society, 1885).*
(2) *WAHRLICH, Report on the Cultivation of Orchids with Rooting of Orchids (Report of the German Botanic Society, 1886).*
(3) *SCHLICHT, Report of the German Botanic Society, 1888.*
(4) *JANSE, The Rooting Endophytes of Some Javanese Plants (Ann. of the Garden of the Buitenzorg, XIV).*
(5) *E. STAHL, Der Sinn der Mycorrhizal Biology (Pringsheim's History, 1899).*

There is no symbiosis or parasitism here, because there are not two individuals, plant and fungus. One, the endophytic fungus, is the element that constitutes the plant. Later, Noël Bernard **(1)** set out to demonstrate that the infection observed by Franck is indeed a symbiosis and that it is the essential condition for the formation of tubers in plants. There seemed to be evidence to support such a hypothesis. Indeed, it had been observed that the tubers of Orchids and Ophrydeas are always and normally infected by filamentous fungi of the genus *Fusarium.*

Noël Bernard concluded from his study on potato tuberculation:

1. That there is, between infection and tuberization, an exact coincidence that is particularly easy to observe.

2. That tuberization is a consequence and a symptom of infection, in the well-defined case of infection of the roots by endophytic fungi specifically related to those of Orchids.

Potato tuberculization would therefore be caused by a mold of the genus *Fusarium (Fusarium solani)*.

We will follow Noël Bernard in his study in order to show the errors that could have led to such conclusions.

First, he notes that it is surprising that, in all countries and in all terrains, the plant is always infected by the same Fusarium solani. He attributed this fact to the propagation of this mold by the tubers that serve as seeds. To this end, he looked for the presence of *Fusarium* in the tubers. Here is what he observed:

Placing well-washed potatoes in a humid chamber, he almost constantly observed the development of *Fusarium* on their surface. He therefore concluded that *Fusarium* always exists in the tubers, but only in the peripheral, sub-epidermal areas.

Here is how he demonstrated that *Fusarium* does not exist inside the tubers:

"*The tubers which carry Fusarium solani, however, appear to be as free from infection as the tubers of Ophrydeae. If fragments of living parenchyma are removed aseptically and left in sterile tubes in a humid environment, no development of Fusarium is generally observed; now, if spores of Fusarium solani are inoculated onto these living and sterile fragments of tubers, this fungus develops there, although poorly; therefore, the parenchyma of the tubers does not normally exist, which appears to be an aseptic environment. I have also kept large fragments of parenchyma of new potatoes aseptic for several months; when working on tubers kept in a cellar for a year, I have only rarely had a development of Fusarium.*"

(1) *Noel BERNARD, Etude sur la tuberisation, Revue G. de Bot., vol. 14, 1902.*

"*Whatever it may be, the presence of Fusarium on the tubers is certain and it does not normally penetrate their mass, which leads me to the conclusion that in this case, as in that of the Ophrydeae, the tubers are normally free from disease.*"

Noêl Bernard explains the formation of tubers by *Fusarium*:

1. By the fact that this mold always parasitizes the roots and that it is always present in the bud which develops to form a tuber.

2. By the fact that *Fusarium* does not exist in other parts of the plant, stem, leaves and fruits.

3. By the fact that there is no formation of tubers in plants obtained by sowing potato fruits or seeds which do not contain *Fusarium*.

These assertions of Noël Bernard are refuted by the facts; here is the demonstration:

1. *Fusarium solani* does not exist only in the sub-epidermal regions of the tuber, but in all its parts and constantly. To obtain it in any region of the tuber, the procedure is as follows: a large potato (sausage-shaped) is carefully washed and dried with a sterile cloth. It is broken into two parts so as not to soil the exposed parts of the parenchyma. With a fine spatula (a steel knitting needle flattened at its end so as to form a thin spatula three to four millimeters wide), the parenchyma is scraped from the center of the exposed surface to reduce it to a pulp (or a piece is removed and ground in a mortar sterile); the pulp obtained is transferred to agar **(1)**.

By making about ten tubes for any region, we always obtain, but often after four days to three weeks or more, the vegetation of *Fusarium solani* in one, two or three tubes. A semi-liquid bacterial culture often develops in the tubes; this is a bacterial culture of *Fusarium* or *Spicaria*; transferred to agar, it can give rise to *Fusarium solani.*

In addition to *Fusarium solani, Spicaria solani* is also frequently found in all regions of the tuber. Other gonidarian forms are also found, discussed later: *Sporothricum, Citromyces*, and *Penicilium* forms, and even *Phytophtora infestans*.

2. *Fusarium solani* exists in the aerial stems and fruits of the potato, as well as in the tubers and roots.

Fruit. *Fusarium solani* is easily obtained by spreading the pulp of potato fruits on agar; this pulp often slowly becomes covered with white molds; they may not appear for 15 to 20 days, or even longer.

(1) *For this purpose, I used agar made with beef broth and containing 0.5 percent glucose and 5 percent maltose; I do not indicate this medium as the most favorable for the experiment cited; it seems certain, on the contrary, that more favorable ones can be made.*

At first, these molds, when examined, appear sterile and contain only a few spores or a few rare *Fusarium* fruiting bodies. Later, the fruiting bodies become abundant.

I obtained various gonidial forms from this fruit pulp, including *Fusarium, Spicaria, Sporothricum*, and *Penicilium solani*.

Stem. *Fusarium solani* is easily obtained by culturing the tissues of the central, aseptic part of potato stems, using the following procedure:

A healthy stem (as large as possible) is cut transversely at the nodes after having washed it well and dried it with a sterile cloth; at one end of each internode, a median slit one centimeter long is started with a scalpel, then, by pulling on each of the two halves, the internode is divided into two longitudinal fragments; the edge of each fragment is free of dirt; with a fine spatula, the medullary part of the internode is scraped to obtain a pulp which is spread on agar. Four or five tubes are thus inoculated with each internode.

Thus, in a quarter or half of the crops, we obtain molds that are mainly *Fusarium, Spicaria, Sporothricum, Citromyces,* and *Penicilium solani*, that is, the same molds already obtained on the pulp of tubers or fruits.

On the potato stem crops, I also obtained another gonidial form that is of great interest and which will be discussed in connection with the study of potato blight: it is *Phytophtora infestans*.

It appears amply demonstrated, from now on, without it being necessary to insist further:

1. That the different molds consistently obtained by culture of the tissues of all parts of the plant, tubers, roots, stems, fruits, are the various gonidial forms of the organic mold specific to the potato.

2. That *Fusarium solani* is not a potato parasite. It is a gonidial form of its organic mold that appears, either on the surface of tubers or in in vitro cultures, by modification of the normal vegetative form that organic mold takes in healthy tissues.

3. That the tuberculization of the potato and of plants in general has no connection with a parasitism that does not exist, nor with phenomena of symbiosis, since there exists, in the tubers and in the entire plant, only a single individual consisting entirely of the specific organic mold; it is this that has been taken for a symbiotic endophytic fungus.

The evidence given is sufficient for demonstrations. There are, however, still others that have not been sought specifically to reinforce the first. Here they are:

1. I have previously explained (*Multiplicity of gonidial forms of molds*, page 301) the relationships that exist between the various gonidial forms of organic mold of the potato and, in particular, *the direct transformation of Fusarium solani into Penicilium solani*.

These transformations make it possible to understand the cause of obtaining, by culture of the same tissue, multiple gonidial forms all deriving from normal organic mold; they explain why none of these gonidial forms observed, either in in vitro cultures or even on the plant in the standing stage, can be considered a parasite in the exact sense of the word.

2. Direct proof of the connection between the organic mold specific to the potato and Fusarium solani was given to me by the observation of a crustare of Fusarium solani growing on the parenchyma of a tuber. Portions of the mold, carefully removed with a fine curved needle so as not to touch the surface of the potato, were placed on a slide for microscopic examination. This examination showed that *the mycelium had carried with it starch grains to which it gave birth.* Examination of the composition of the starch grains immediately confirmed this mode of formation; *these grains were perithecia formed by the Fusarium mycelium.*

Then referring to a preparation obtained by scraping the parenchyma of the tubers, I easily observed that the starch grains it contains have the same origin and the same mode of formation.

Subsequently, I observed that this fact is constant in cultures of *Fusarium* growing on potatoes.

Fusarium solani is therefore indeed a gonidian form of the organic mold specific to potatoes since it retains an essential physiological property of this mold, that of producing, like it, starch grains identical to those that exist in the parenchyma of the tubers.

Because of the importance of this question, it was the subject of a study that I will present.

Formation of starch grains by the mycelium of Fusarium solani in crops and by normal organic mold in the parenchyma of tubers.

We will first examine the formation of starch grains by the mycelium of *Fusarium solani*. Plates 142 (Figs. 1, 2, 3, 4) and 143 (Figs. 1, 2, 3, 4) contain photographs taken of a preparation of *Fusarium* that has grown on a potato.

Let us first examine Figure 4, Plate 142.

It shows a mycelial branch A whose ramifications are laden with starch grains; it is also easy to see that these grains are perithecia formed by the coiling and curling of the mycelial branches. There is no doubt that my- celial branch A belongs to *Fusarium solani,* because some of its branches bear fruiting bodies of *Fusarium* and, on the other hand, these are noticeable on large filaments running along the surface of the perithecia and even inside them.

The perithecia are made up of a single or several branched mycelial filaments, all of whose branches coil and curl together to form the ovoid mass of the starch grain. Starch grains are known to grow and are found in all sizes in the potato pulp. Starch grains grow because the entire branched mycelial branch constituting the perithecia grows and continues to develop.

The same branched filament forms a series of starch grains. Fig. 2, Plate 142, shows a group of four grains formed by the ramifications of the same mycelial filament hidden at the top of the figure: two large ones, A, B, and two smaller ones, C, D, arising on the latter. In reality, it is the same grain or the same perithecia that constitutes the entire mass; the apparent separation into distinct grains results from the constriction of the perithecia at certain points, either by a mycelial filament creeping on the surface, or by a foreign filament forming a collar that constricts the mass.

Mass F in the same figure 2 constitutes, in the same way, three grains belonging to the same perithecia. In figure 1 of the same plate we see a perithecia constituting five large grains and two smaller ones A, developing on their surface.

This constriction of the perithecia is the cause that gives certain starch grains, such as those of grass seeds, for example, their irregular and bizarre shape.

The constitution of the starch grains is very clear in mass A, B, of figure 2; we see the ramifications of filament E spreading out in the mass. In grain A of figure 3, we can distinguish the coiling and winding of the mycelial ramifications of the initial filament.

This constitution must be studied with a magnifying glass to be clearly perceived; this examination of the enlarged figures also shows an important particularity; we can observe on the interior filaments of the grains, at certain points, fruiting bodies of the *Sporothricum* type, that is to say, constituted by a pedicle of variable length bearing a round or oval terminal spore; these fruiting bodies exist in many grains in figures 1, 2, 3, 4, of plate 142; we will see them very clearly in grain F of figure 2. In figure 4, plate 143, we note both, in a grain, the fruiting bodies of the *Sporothricum* **(1)** type and those of the *Fusarium* type. Finally, we can, by following the isolated mycelial filaments of *Fusarium*, find the two species of fruiting bodies *on the same filament.*

This fact is important because the *Sporothricum* type is the normal fruiting type of the organic mold that constitutes the starch grains in potato tubers and which we will now study in the tubers themselves. Figure 5, Plate 143, represents a preparation, stained with dahlia, of the liquid obtained by scraping potato pulp. It shows the identity of shape of the starch grains with the grains formed by *Fusarium solani* in vitro and represented in Plate 142; fruiting bodies of the *Sporothricum* type abound there, and it is found that they are identical to those contained in the grains formed by the mycelium of *Fusarium solani.*

In preparations of scraped potato pulp, stained with dahlia, large branched mycelial trunks are easily found, the branches of which bear large masses of perithecia, or starch grains, to which they give rise. Figure 2, Plate 144, shows a mycelial branch of this kind. The mycelial ramifications starting from the large trunk and entering the perithecia they constitute are easily distinguished.

The identity of the mode of formation of starch grains, either by organic mold in the tuber or by the gonidial form Fusarium vegetating freely on its surface, is clearly apparent by comparing Figures 2, Plate 144, and 4, Plate 142.

Starch grains are formations identical to the perithecia that constitute the pulp of fruits, such as an apple or a pear, and also identical to the perithecia constituting the fatty tissue of animals.

In all these formations, the finest ramifications of the formative mycelial branch bear short spore-bearing appendages and spiral appendages, whether it is an animal or a plant organism. It is a bushy arborization, whose fine ramifications bear appendages of this kind, which constitutes the entire mass of these formations.

I investigated whether the formation of starch grains is a special property of the organicmold of the plant that only manifests itself in the tubers. I looked for starch grains in the stem; it also contains them, although in very small quantities relative to the tubers, and they form there in the same way.

We can conclude from these observations relating to the formation of starch grains:

1. That these are formations (perithecia) of the organic mold specific to the plant.

2. That *Fusarium solani*, the gonidian form of this organic mold, retains, when it develops on the potato, the same property of forming starch grains.

(1) *I refer this fruiting type to the Sporothricum type, although it differs from it, because it is the one to which it appears to be closest. I have not found this type in the existing nomenclature.*

3. That tuberization is the manifestation, at certain points of the plant, of this property of the specific organic mold pushed to a high degree.

If we could believe in a symbiosis between higher plants and parasitic endophytic fungi, it is because, by cultivating roots or tubers, we obtained these forms of fungi and from this we deduced parasitism.

Now, it is proven in this work, by a whole series of studies whose results agree, that a plant is an organized mold and that the specific mold that constitutes it, transported to artificial nutrient media, develops in new and multiple gonidial forms.

What we considered to be parasitic endophytic fungi are these gonidial forms of the specific organic mold; they were obtained because we made the plant tissue vegetate under abnormal conditions.

The tuberization of plants, which might seem to be an argument in favor of the symbiosis of higher plants with parasitic endophytic fungi, *is simply a normal physiological property of the specific organic mold;* the parasitism invoked does not exist and neither does the symbiosis, since molds, considered as *normal endophytic* parasites, are gonidial forms of the specific organic mold.

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Bacterial plant diseases

Study of bacterial wet rot of potatoes

Only this single bacterial disease will be studied here because, being so widespread, it makes it easy to establish the causes and true nature of bacterial plant diseases in general.

During the months of August and September 1924, and in the course of the study I was continuing in the countryside on potato tuberization, I noted that a large number of tubers were affected by wet rot. The tubers, carefully removed from the soil, showed an intact epidermis, although in certain points their parenchyma had been transformed over a large area (width and depth) into a mush similar to very soft potato puree or even into a cloudy, stringy, cream-colored liquid. This mush and liquid, examined under a microscope, were made up of an enormous quantity of bacilli and granulations of various sizes (Fig. 1, 2, pl. 145).

This bacterial rot has already been studied by many observers; but it is impossible to draw a precise conclusion from these studies, because around ten bacterial species have been indicated as being able to cause it. I quote here on this subject a few passages from the book by Delacroix and Maublanc **(1)**.

(1) *DELACROIX AND MAUBLANC, Diseases of Cultivated Plants, page 50, Baillère et Fils, Paris 1916.*

''I must say immediately that this wet rot is not the work of a single bacterial species, is not a disease due to a specific, determined bacterium; moreover, the various organisms that can be extracted from the rotting pulp are not capable of attacking a healthy tuber, at least in the vast majority of cases...''

''Van Tieghem was the first to attribute very clear cases of rot with butyric acid production to the *bacillus amylobacter*; according to Von Hall, this bacterium does exist, but the deterioration should not be attributed to its action alone; other bacilli would even play the main role, and in particular *Bacillus solaniperda*…''

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"However, in many cases, wet rot in potato tubers is caused by other bacteria, particularly those that we previously identified as capable of parasitizing potatoes: Bacillus solanincola, solanacearum, fluorescens, liquefasciens, phytophtorus, atrosepticus; they act alone or, more often, aided by common species to which they have opened the door..."

According to this presentation, around ten different bacteria could cause this disease, which would be, in short, an attack on the tubers by various bacterial parasites. This question, which thus appears very complicated, is in reality simple.

I indicated above that the soft slurry that constitutes wet rot is made up of bacilli and granulations of various sizes; the starch in the parenchyma has disappeared.

One might believe, through microscopic examination, that this slurry contains several bacterial species, notably several bacillary species and several *species of micrococcus.* But we know, from the facts indicated in the chapter *Evolution of Bacterial Cultures,* that these different bacterial forms can belong to the same species and that they are the segmentation elements of the mycelium or the spore-forming elements of molds.

Indeed, the bacilli and granulations existing in the rot slurry are *the bacterial and spore -forming elements of the organic mold of tubers.*

It is easy to obtain a pure culture of *Fusarium solani*. It happens that such a culture, subcultured on agar, does not give rise to aerial mycelium; it can develop in the form of a smooth, non-villous or even semi-liquid material, in which the mycelial elements of the *Fusarium* ramify.

Examining this veil, we see that it contains, in addition to the normal elements of the *Fusarium*, a considerable mass of the bacterium contained in the wet rot and, in addition, identical spherical granules. If we follow a mycelial branch, then the fine ramifications which it emits, we see that these have, at their extremities, only a width equal to that of the bacteria and we see that a certain number of them are in the process of fragmenting into bacteria. This is a direct observation of the nature and origin of the

wet rot bacteria of the potato; they are *the segmentation elements of the fine terminal mycelial ramifications of its specific organic mold.*

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Conversely, and as a counter-test, the characteristic gonidial forms of organic potato mold can be reconstituted by cultivating on agar either the rotten pulp or the pure bacterial culture that can be obtained from it.

Many tubers are rotten to the central region; from one of these tubers, the epidermis covering a rotten region is carefully removed over an area of five to six square centimeters; with a sterile knife, without stirring the mass, the rotten slurry is gradually removed (flaming it after each removal) to the center of the tuber; then, in this region, a little of this slurry is taken and cultured on several agar tubes; after a time varying from about four to fifteen days, this slurry gives rise exclusively to the gonidial forms of organic mold: *Fusarium solani, Spicaria solani, Citromyces* and *Penicilium solan*i, etc. No other mold appears; if a bacterial species foreign to the tubers existed in the slurry, one would surely observe the appearance of one of the gonidial forms of hyphomycetes which are specifically related to it.

Finally, rigorous proof of the spontaneous formation of wet rot bacteria by the specific organic mold of potatoes is given by the following experiment:

1. Healthy, smooth potatoes with few, very shallow eyes into which the bristles of the brush can penetrate are carefully washed under running sterile water and brushed; they are dried and some are placed for twelve hours in a glass vase surrounded by a cooling mixture of crushed ice and sea salt; other potatoes are placed for two hours in sterile water heated to 55°. The potatoes thus treated are carefully dried with a sterile cloth and the two batches are placed separately in two sterile, closed crystallizing dishes. After three or four days, it is found that the potatoes in both batches are affected by characteristic wet rot. This rot contains bacillary elements identical to those of spontaneous rot of tubers in the earth.
Plate 145 represents:

1. In figures 1 and 2 the usual elements of bacterial rot of the tubers that have just been extracted from the earth.

2. In Figures 3, 4, and 5, the elements of bacterial rot of healthy potatoes, subjected to frost for twelve hours.

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3. In Figures 6 and 7, the elements of bacterial rot of healthy potatoes heated for two hours at 55°C.

4. In Figures 9, 10, 11, and 12, the bacillary elements of a pure, poorly developed, semi-liquid culture of *Fusarium solani* obtained by culturing healthy potato pulp.

In wet rot of the potato, bacilli of various sizes are found, mainly an enormous bacillus with a width of 3 to 10 μ and a much finer bacillus (0 μ 4 to 0 μ 5 wide on average) and of variable length according to its state of segmentation. The origin of these two forms is easy to find; the larger (Fig. 1, 2, pl. 145) comes from the segmentation of the first abnormal mycelial vegetation of the organic mold in transformation; this vegetation gives rise either to a mold of the *Phytophtora* type, or to *Fusarium solani*, or, if it segments to pass into the bacterial form, to the large bacterium of 3 to 10 μ wide; the small bacterium corresponds to the mycelial segmentation of the fine mycelium of the *Fusarium* type.

These two characteristic forms are found both in the wet rot of potatoes frozen or heated to 55°C and in spontaneous wet rot in the soil.

This evidence is further supplemented by the following fact: if either the pulp from the center of a healthy potato or the scraped tissue from the center of the healthy aerial stem is cultured in a sugar agar tube, in certain cultures a thick, creamy material develops, which slowly flows to the bottom of the tubes; this creamy material contains the bacillary elements characteristic of potato rot, or reproduces them if it is inoculated in sugar peptone broth. Figure 8, Plate 145, represents the bacillary forms obtained by culturing the healthy tissue of the aerial stem taken 20 centimeters above the ground.

The main fact, highlighted by these demonstrations, is of considerable importance with respect to the etiology of animal and plant diseases, because it demonstrates:

1. *That the bacteria whose presence is observed in living organisms, healthy or diseased, do not necessarily come from an external infection.*

2. *That these bacteria can easily arise spontaneously in these organisms and that the element which gives rise to them is the specific organic mold.*

In this specific case, the primary cause of the disease is therefore not the bacteria present in the diseased organs, but the set of conditions that determined the transformation, into a bacterial culture, of certain elements of the specific organic mold. There are therefore autogenous diseases that spontaneously develop bacterial germs.

From this study of wet rot of the potato, it follows:

1. That, the bacterial elements (bacilli and granulations) observed in the rot are elements originating from the organic mold of the potato.

2. That these elements are not the initial cause of the disease and are only a result and a sign of it.

3. That the initial cause of the disease is the one that determined the transformation of the organic mold into bacilli and granulations. This cause is well known to growers: it is a persistent excess of soil moisture (too frequent or prolonged rain, marshy soil).

It is therefore illusory to seek to sterilize the soil or to burn diseased organisms to destroy bacterial elements, since these form spontaneously in healthy plants and are not the initial cause of the disease; the means used to destroy bacterial germs are fatally ineffective; only a treatment opposed to the initial cause, and having the effect of increasing the resistance of plants to this cause, can have a favorable effect.

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Potato Diseases

Potato diseases whose cause is attributed to parasitic fungi.

The most widespread of these diseases are:

1. Potato late blight, attributed to *Peronospora infestans.*
2. Yellow disease (*Verticillium wilt*) attributed to *Verticilium alboatrum* (Remecke and Berthold).
3. Brown spot disease, attributed to *Alternaria solani* (Sirauer).
4. Rhizoctonia scab and collarette or cuff disease, attributed to *Hypochnus solani* (*Corticium solani* - Prilleux and Delacroix).
5. Common scab attributed to *Actinomyces*.
6. Powdery scab, attributed to *Spongospora subterranea.*
7. Varicose black scab attributed to *Synchytrium endobioticum* (Schilb).

* * *

I will not make a successive study of these diseases here; they will be treated as a whole, because *the series of fungi, considered to be the parasites which cause them, is that of the gonidial forms of the organic mold specific to the potato.*

Healthy, normal specific organic mold, cultivated on artificial media, gives rise to a whole series of gonidial or vegetative forms, including *Phytophtora infestans, Verticilium alboatrum, Alternaria solani*, etc., considered as parasites.

There can be no doubt that these forms are closely related to each other and that they are indeed only varied gonidial forms of the specific organic mold of the potato, because here the observer has at his disposal three different, reliable methods that he can use as a means of control:

1. Direct transformation by culture on varied artificial media. A gonidial form taken directly from the plant and inoculated on various artificial media undergoes changes in form. Two very different original forms can transform into a common form, the *Penicilium* form, for example.

2. The transformation of a gonidial form into a bacterial culture (culture in liquid media) followed by its transformation into hyphomycetes (transfer of the bacterial culture to solid media).

3. The transformation of a gonidial form into other forms by inoculation into a guinea pig and culture of its organs.

Using these various means of study, all gonidial forms can be easily transformed into *Penicilium*, which can serve as a control for each of them. Plates 272 and 273 contain the various types of *Penicilium solani* obtained by inoculating healthy potato pulp into a guinea pig or developing spontaneously on the pulp of tubers. From the spontaneous development of these gonidial forms on healthy plant tissue, we can conclude:

1. That the various molds, considered until now to be parasites of foreign origin brought into the plant by infection, do not have this origin; *they arise spontaneously there* and the element that gives rise to them is the normal specific organic mold. This fact cannot be doubted, being a general law for all animal and plant organisms.

2. These molds are not the initial cause of the disease; they are only the result and the witness. The specific organic mold only abandons its normal vegetative form to take on an abnormal and pathological gonidial form when it is suffering and affected by unfavorable living conditions.

3. The initial cause of the disease is that which puts the specific organic mold in a state of suffering and causes the change of its vegetative form.

These initial causes, determined until now to be excess or insufficient soil moisture, poor nutritional conditions related either to insufficient or poor fertilizer selection, or to a defect in the chemical makeup of the soil, are indeed the real causes of potato diseases.

In a brochure on potato diseases by Ducomet and Foex, the initial cause of these diseases is discussed. Here are some passages on this point **(1)**:

"*It was long believed that degeneration, which results in a progressive decrease in productivity, was inevitable.*

(1) *DUCOMET AND FOEX, Potato Diseases, Publication of agricultural offices, "Maison Rustique" bookstore, 26, rue Jacob, Paris.*

It is still commonly accepted that it is either the ageing of the variety, or a state of misery caused by defective growing conditions, or prolonged cultivation in the same environment without renewal of the plant, or the debilitating action of repeated attacks of certain diseases (potato blight, for example).

"*On the other hand, precise observations and rigorous, methodically conducted experiments have revealed that these are in reality infectious, contagious, hereditary, incurable diseases, the severity of which increases with the generations*."

We have just seen that these diseases are not caused by parasites, in the true sense of the word, since the molds to which this qualification has been given arise spontaneously on plant tissues in a state of suffering or degeneration; since the disease can develop spontaneously, it is therefore not permissible to give it the qualification of infectious.

Indeed, the experimenters who carried out infection experiments were unaware of the existence of the spontaneous formation of germs that they considered infectious; that rot bacteria and *Peronospora infestans*, for example, arise spontaneously on plants and that the disease, thus developing spontaneously, does not recognize as its original cause an infection by this vegetative form.

The transmission of a disease from one subject to another by infection may exist, but it will only be possible to make a judgment on the reality and importance of this infection after new contamination tests in which care has been taken to eliminate all causes of error linked to the possibility of spontaneous development of the disease in the subjects subjected to the infection.

Until this determination is made, it must be considered that potato diseases *are not infectious and contagious diseases and that they result from a state of misery determined by defective cultural or meteorological conditions.*

Potato diseases whose causal agent has not been determined.

These diseases are: Crinkle and mosaic; Leaf roll; Variegated leaf spot;

Much research has already been done to determine the causes of these diseases. The preceding explanations explain the futility of this research. When a tissue decays, it gradually loses its fundamental physiological properties, including that of producing chlorophyll grains; it turns yellow, then becomes ochre and brown. In the case of the diseases listed above, decay occurs in localized areas, without the formation of mold on the surface of the plant.

Knowledge of the nature and constitution of plant tissues indicates that the nature of these diseases does not actually differ from that of diseases characterized by external mycelial vegetation, *the latter being only an external expansion of the diseased organic mold*; the latter, in a state of suffering, progressively loses its chlorophyll (mosaic and variegation) or vegetates in an abnormal way; the zero growth in certain points, persistent or exaggerated in others, has the effect of determining the curling and the rolling. These facts explain why only the usual anatomical elements are observed in the diseased regions.

The demonstration of the facts stated so far could only be complete and decisive by the *spontaneous reproduction, starting from healthy tissues, of the various types of molds considered until now as the parasites which are the infectious agents of potato diseases.*

This demonstration, made in the following chapter, establishes that all molds, considered to be parasites of the potato, *are gonidial forms of its specific organic mold.*

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Gonidial forms of organic mold specific to potatoes. Evolution of this mold when cultivated on artificial nutrient media.

The main fungi considered to be the parasites that cause potato diseases are:

1. *Phytophtora infestans* (late blight).
2. *Fusarium solani* (dry rot and mummification of tubers).
3. *Verticilium alboatrum* (yellow disease).
4. *Alternaria solani* (brown spot disease).
5. *Hypochnus solani* (cuff or collar disease, and smallpox or rhizoctonia scab of tubers).
6. *Synchytrium endobioticum* (warty scab).
7. *Spongospora subterranea* (powdery scab).

The question of the specificity of the first four fungi in the diseases they cause can be easily judged by culturing the healthy tissue of the aerial stem of the potato.

The procedure used for this culture has been described previously (Page 327); it is easy to do while avoiding any contamination of the tissue scraped from the center of the stem, if it is large enough. Healthy stems are chosen from a field where the individuals are free of disease and from plants spaced apart. Cultures made on very sweet agar (5% maltose, 5% glucose) are kept at room temperature, in the light.

After three to five days (sometimes six or eight), colonies of molds of the following types are seen developing on the cultured tissues:

1. *Fusarium solani*.
2. *Spicaria solani*.
3. *Monosterigmata*.
4. *Verticilium solani* (V. alboatrum).
5. *Phytophthora infestans.*
6. *Alternaria solani.*
7. *Penicilium solani.*

Figs. 1, 2, 3, of plate 146, represent three photographs of colonies of *Phytophthora infestans* born on cultured tissues of the healthy stem.

Examination of the gonidia contained in these photographs leaves no doubt about their identification. Figure 1 contains, in addition to the isolated gonidia, several of the characteristic filaments (*sporiferous hyphae*) which often carry a series of them.

Fig. 7 represents *Spicaria solani*, which is obtained more frequently than *Phytophthora*.

Figures 8, 9, 10 represent various aspects of an Alternaria solani culture. This is only a form of the *Fusophragmium* type; like this, it represents a stage of development towards the *Penicilium* form.

Verticilium solani (*Verticilium alboatrum*) is represented in Figs. 3, 4, 5, Plate 263, and 8, 9, 10, Plate 276). In the study of foot-and-mouth disease and vaccinia, whose pathogen is the organic mold of the potato, I obtained, from the serosity of the canker sores and the vaccinal virus, *Verticilium alboatrum*, which is easily transformable into other characteristic gonidial forms.

As for *Fusarium solani*, it has already been represented in Plate 143.
Penicilium solani is, on the other hand, represented in Plates 272, 273.

Thus, by culturing the healthy, uncontaminated tissue from the center of the aerial stem of the potato, we see the spontaneous growth of the fungi that have been considered to be specific infective parasites of these diseases:

Phytophtora infestans, which is believed to cause potato blight.

Verticilium solani, which causes yellow disease.

Alternaria solani, which causes brown spot disease.

The autogenous *formation* of these molds on healthy tissues therefore indicates that they are gonidial forms of organic mold in the plant, *therefore spontaneous in the plant, and not brought by infection;* they are therefore a sign of the disease, but not its determining cause.

I have, in addition, obtained two other interesting gonidial forms by culturing the central tissue of the potato stem.

1. A mold shown in Fig. 5, Plate 146, which appears to be an evolutionary form of organic mold towards the *Aspergillus* form; it forms rare *Phytophtora gonidia* (Fig. 4, Plate 146); its gonidial stems possess certain characteristics of the *Verticilium* form,

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but are clearly distinct from those of *Verticilium alboatrum*. I obtained this same gonidial form by transforming cultures of tetanus, a disease whose pathogen is organic mold of the potato.

2. A monosterigmat form of the type *Acremonium alternatum* (Fig. 6, pl. 146).

If, instead of the stem, the pulp obtained by scraping or crushing a piece of parenchyma taken from the center of a fruit or the center of a healthy tuber is cultured on agar, the spontaneous formation of the following types of mold is observed in the cultures:

In fruit cultures: *Fusarium, Spicaria, Sterigmatocystis*, and *Penicilium solani.*

In cultures of healthy tubers: *Phytophtora infestans, Fusarium, Spicaria, Monosterigmatis, Aspergillus, Sterigmatocystis, Citromyces, Penicilium solani.*

By culturing the pulp of regions of a tuber affected by brown rot, I obtained a well-characterized gonidial form (Fig. 7, pl. 148) which appears to be a form of evolution of *Verticilium solani* (*Verticilium alboatrum*) towards the *Penicilium* form. This mold can, in fact, be considered a Verticilium. Its culture was transformed over time into the *Penicilium* form. This mold is very interesting because I obtained it by transforming a bacterial culture of the pathogenic agent of foot-and-mouth disease, an agent which is the organic mold of the potato.

In agar cultures of the pulp obtained by scraping the parenchyma of healthy potatoes, a white mold forms, generally sterile at first; the fruiting bodies appear after a variable time; it was only after one to two months that I saw *Phytophtora* fruiting bodies appear.

One should not deduce from the absence of fruiting bodies on a mold, or even from the absence of mold in a culture, that none will form; these investigations must be conducted with great patience; a sufficient number of tubes must be inoculated and *kept for a very long time;* it is, in fact, after several months, some- times six months or a year, that the most curious spontaneous transformations of molds are observed.

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This is how I obtained a very fine culture of *Phytophtora*; this culture reproduces on sugar agar and has retained its type until now; the very abundant gonidia are formed,

either in groups at the end of branched filaments, or singly at the end of a filament, or along the branches (Fig. 6, 7, 8, pl. 149).

Thus, Phytophtora infestans arises spontaneously in vitro on both healthy tuber tissues and those of the stem.

These facts therefore establish that the molds that have been considered parasitic fungi, causes of potato diseases, *are gonidial forms of organic plant mold and can all appear spontaneously at any point on the plant.*

Other demonstrations further support the decisive proof that has just been given; they are provided:

1. By the direct, spontaneous transformation of the *Phytophtora* type into the *Fusarium* type, and of the *Fusarium* type into the *Penicilium* type.

If we break a diseased potato into two parts with the dead, brown areas attributed to *Phytophtora* and follow the mold from its appearance, we notice:

A. That at the beginning it is constituted by a mycelium with large filaments whose articles fragment very easily (Fig. 1, 2, 3, 4, 5, 6, pl. 147). These constitute the enormous bacterium previously described in wet rot. This is indeed *Phytophtora infestans*, because characteristic gonidia are found in the preparations (Fig. 6, pl. 147). In addition, very large gonidia are found stuffed with large spores (Fig. 3, pl. 147). Most of the mycelial articles are, moreover, filled with the same spores of endogenous formation (Fig. 2, 3, 4, 5). It is this form that H. Pethy Bridge appears to have described as a new species (*Phytophtora erythroseptica*, Pethy Bridge, Nov. Spec.) **(1)**. There is only one form here, not a species; moreover, there cannot be several species of *Phytophtora infestans*, this being only a vegetative form, external to the plant, of its organic mold and not being able itself, for this reason, to constitute a species.

Phytophtora infestans is, moreover, only a rudimentary type of the *Peronospora* form of the organic mold of the potato. This form, which is confused with the *Botrytis cinerea* form, is represented in Figure 1, Plate 301 bis. It can be obtained by culturing the organ pulp of a guinea pig inoculated with the juice of the tuber parenchyma or by placing potato leaves in a humid crystallizer maintained at 24-26°.

It has already been observed that, in tuber lesions, the mycelium of Phytophtora does not persist and is replaced by that of other species, notably Fusarium and Spicaria solani.

(1) *Pethy Bridge, The Scientific Proc. of the Royal Dublin Society, 1913, p. 530.*

The explanation for this fact is provided by the daily examination of a colony of *Phytophtora*; *after a few days, fine filaments bearing fruiting bodies characteristic of Fusarium solani are seen to be born on the large mycelial filaments of the latter* (Fig. 2, pl. 148).

B. That when observing the development of *Fusarium solani* cultures in vitro, a characteristic and progressive transformation is quite frequently observed (Fig. 1, 2, 3, 4, pl. 148); round spores identical to those of *Penicilium solani* develop externally on the mycelial filaments; later, certain *Fusarium* filaments give rise to branches ending in the fructifications of P*enicilium solani* (Fig. 4, 5, pl. 148).

The transformation is marked by the appearance of a green spot (*Penicilium* colony) on the edge of the cream-colored culture of *Fusarium solani*.

2. By the direct transformation of *Phytophtora infestans* into *Mucor solani*, and of the latter into *Penicilium solani.*

Figures 1, 2, 3, 4, plate 149, are photographs of a culture of *Phytophtora infestans* in the process of transforming into the *Mucor* type; the transformation occurs either in the original culture itself or on the culture transferred to agar in vitro (Fig. 5, 6, 7, plate 149); at certain points (Fig. 5 and 6) the mycelium forms some fruiting bodies identical to the gonidia of *Phytophtora*. This *Mucor* form gives rise to *Penicilium solani* when it is passed through the organism of the guinea pig.

3. By the spontaneous transformation of the moist rotten pulp of tubers into mold possessing both fruiting bodies of the *Fusarium* type, others analogous to those of *Phytophtora*, and finally some formations of the *Alternaria* and *Ovularia* types.

For this transformation, I spread on agar the wet rotten slurry of the tubers containing no elements other than those contained in Figures 1 and 2, Plate 145; it is this slurry that was used to establish the photographed preparations.

Figures 7, 8, 9, 10, Plate 147, contain both gonidia of the *Phytophtora* type and fruiting bodies of *Fusarium*, sometimes on the same mycelial filament. Figure 7 contains forms of *Alternaria solani*. Finally, some gonidia take a form that has not been described; they are bi- or tri-locular and often echinulate.

This variability of fruiting forms in the same crop shows how little value the characters that have served to distinguish a large number of species by the form of their fruiting bodies have. The origin of these forms can easily be found by examining the composition of the parenchyma of plants; in a green potato leaf, one will easily find elements identical to the gonidia of *Phytophtora* or the string-like dilations of *Alternaria*, or the fruiting bodies of *Hypochnus solani*.

* * *

By applying to the study of tuberization and potato diseases the concepts provided by the study of the constitution of plant tissues, it has been successively demonstrated:

1. That potato tuberization is not due to the parasitism of an endophytic fungus, *Fusarium solani*, and that it is carried out by the organic mold specific to the plant, of which *Fusarium solani* is a gonidian form.

2. That wet or bacterial rot of the potato results, not from an infection by a heterogeneous bacillus, but from the transformation of organic mold into its bacterial vegetative form under the influence of unfavorable cultural or meteorological conditions.

3. That, in a plant organism, organic mold can spontaneously give rise to bacterial vegetation, under the influence of external causes that put it in a state of suffering (the action of frost and excessive heating).

4. That the bacterial forms of the culture thus developed are indeed those of the organic mold of the plant or its gonidial forms.

5. That the in vitro culture of healthy plant tissues gives rise to the same bacterial forms as the spontaneous rot of plants in culture.

6. That the culture of bacterial rot, carried out in certain conditions, achieves the reconstitution of the gonidial forms of organic mold.

7. That the culture of healthy plant tissues spontaneously gives rise to molds that have been considered parasitic and infective fungi: *Phytophtora infestans, Spicaria solani, Fusarium solani, Verticilium alboatrum, Alternaria solani*, etc.

These facts demonstrate that potato diseases are not due to parasitic and infective fungi and are not contagious. They are the manifestation of the state of physiological misery caused by defective cultural conditions or by unfavorable meteorological conditions.

The various types of mold that appear in diseased regions are abnormal transformations of organic mold, that is, autogenous molds. They are a sign of the disease, but not its initial cause.

The disease does not necessarily manifest itself through its external vegetation in a deviated form; it also manifests itself, simply, by the death of the tissue (curling, rolling, mosaic, browning).

This explains why it has never been possible to find in the tubers, at the time of planting, the spores or gonidia of the molds incriminated as the cause of the diseases that

will subsequently affect the plant or vegetation; they have not been found because they do not exist, the diseases being spontaneous.

The use of antiseptics to destroy the spores of parasites therefore has no reason to exist and no effective action can result from it.

The damage caused to plants by these diseases can only be avoided by improving growing conditions and by means that reduce the influence of unfavorable weather conditions.

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V. Vine Diseases

The various vine diseases are, like those of the potato, spontaneous diseases, due to defective growing conditions and especially to unfavorable weather conditions; the lesions that characterize these diseases are due to a deviation in the form of the diseased organic mold and not to parasitic fungi; in fact, *Plasmopara, Botrytis cinerea*, *Oidium*, and *Guignardia* are gonidial or vegetative forms of organic mold in the vine.

The bacteria reported in certain diseases, *Bacillus uvæ* (Guggini and Macchiati**,** *Bacterial Grape Stain*) and *Bacillus vitivorus* (Baccarini, *Bacillary Gum Disease*), are bacterial forms of organic vine mold unrelated to the initial cause of the disease. Here, as with bacterial diseases of potatoes, there is no infection, the formation of bacteria being spontaneous; they are only a witness to the decay of the organic mold and not the cause of this decay. Only three of the main vine diseases will be discussed here: mildew, gray mold of grapes, and black mold.

Downy mildew and gray rot of grapes.

Downy mildew is caused by *Plasmopara viticola*, gray rot of grapes by *Botrytis cinerea*. I have combined these two diseases in the same description because these two molds are identical: *Plasmopara* is *Botrytis* and vice versa. This identity cannot be in doubt, for it is very easy to see that, in the same clump of fruiting bodies, the two forms attributed to *Plasmopara viticola* and *Botrytis cinerea* exist side by side.

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Already, in the drawings of these two molds **(1)** one realizes either the fantasy of these representations, or the analogy that exists between these molds. In figure 100 of the treatise by Engler and Prantl, part of the fruiting bodies (on the left) can be taken for *Botrytis cinerea,* while the other part would be *Plasmopara.*

(1) *See on this subject: ENGLER AND PRANTL, The Natural Plant Families, etc., fig. 100 and 226 (C).*
DELACROIX AND MAUBLANC, Parasitic Diseases of Cultivated Plants, pp. 103 and 249.
DUGGAR, Fungus Diseaser of Plants, figures 53 and 74.

This difficulty of appreciation results from the fact that the drawings of the molds are often far removed from their form, represent them poorly, and that by schematizing the representation, the author distorts it. A description can only be exact and avoid all confusion if it *is supplemented with photographs.*

Botrytis cinerea is easily obtained by placing a bunch of healthy grapes in a small covered crystallizing dish. With a needle with a curved tip, remove a few tufts of the mold and place them on water (four or five drops) placed on a glass slide; hot drying, dahlia staining and hot water washing. In these preparations we note, at the same time, the presence of the long and robust gonidiophores of *Botrytis cinerea* (Fig. 1, 2, pl. 150) and shorter gonidiophores (Fig. 3,) analogous to those which have been described for *Plasmopara.* In addition, we note the formation of sessile gonidia on all the medium and fine mycelial ramifications (Fig. 3 and 6, pl. 150). At the end of some gonidiophores or isolated filaments, we frequently note a ball-shaped fructification analogous to that of a *Mucor* (Fig. 2 and 3).

The identity between *Plasmopara viticola* and *Botrytis cinerea* is also established by the following facts, which further demonstrate that the unique mold bearing these two names is a form of organic vine mold.

1. Both molds, cultivated on sugar agar and replanted every eight days, spontaneously give rise to the *Penicilium* form of organic vine mold (Fig. 6, 7, pl. 150).

2. If the periphery of healthy grape berries is flame-sterilized, and a fragment of pulp taken from the center of the berry is inoculated onto agar, the cultures produce either the *Penicilium* form of organic vine mold, or the *Plasmopara (or Botrytis)* form, or both forms at the same time.

It follows from these facts:

1. That *Plasmopara viticola* and *Botrytis cinerea* are one and the same mold.

2. That this mold is not a parasite: it is a gonidial form of organic vine mold.

3. That the initial cause of mildew and grey mold is not the mold observed on the leaves, stems, or grapes; this is only a symptom of the disease, the immediate cause of which lies in unfavorable weather conditions, notably excess humidity.

4. That the use of parasiticidal substances against these diseases is not justified and cannot have any beneficial effect, because it has no influence on the initial cause which provokes them.

Botrytis cinerea develops, mycologists say, on most decomposing plants. When the leaves of various plants are left to rot, a mold that appears to be the same develops; but,

examined carefully, it is seen that it differs for each plant species and that for each of them it constitutes a form analogous to that designated *Phytophtora* for the potato, *Peronospora schachtii* for the beet, *Sclerospora* for grasses, and *Plasmopara* for the vine; we will see later that, for lettuce, it is confused with *Bremia lactucae.*

Careful examination of each form allows it to be differentiated from the others.

These facts are put beyond doubt by *the origin of Botrytis in each species.* Indeed, if we examine certain gonidiophores of very distant species, such as those of the *Botrytis* of the vine (Fig. 1, pl. 150) and those of the *Botrytis* (Fig. 1, pl. 151) of lettuce, we see that they are similar; this identity results from the fact that the gonidiophores of the *Botrytis are the mode of terminal ramification (branching) of the mycelial filaments which constitute the parenchyma of the tissues.* By finely dissociating with needles the parenchyma of a healthy leaf in a drop of water, we are able to isolate branched mycelial filaments (emanating from the veins) rigorously identical to the gonidiophores of the *Botrytis*;

The demonstration of these facts will be found in figures 1, 2, 3, 4, of plate 153, which contain mycelial filaments isolated from the parenchyma of a healthy leaf of Althæa; one of them (Fig. 1) is rigorously identical to a gonidiophore of *Botrytis.*

Botrytis, Peronospora, Sclerospora, Phytophtora, etc. are therefore the vegetation of the normal mycelial filaments of the parenchyma developed outside the plant tissues.

Let us add, moreover, that the gonidia of *Botrytis* or of all the molds classified in the *Peronosporaceae* are homologous to normal parenchyma cells, just as the stalk of the gonidiophore and its ramifications are homologous to a sieve tube.

Black rot of grapes.

This rot is attributed to *Guignardia bildvellii.*

On grape berries affected by black rot, pyenidia and spermogonia develop and, in addition, certain pycnidia emit a gonidial form which is of the *Sporothricum* type.

Guignardia bildvellii is a deviation in form of the organic mold of the vine, as demonstrated by the following facts:

1. If a little of the dissociated contents of the pycnidia are removed with a wet tip and cultured on sugar agar, the gonidial form of *Sporothricum* specific to certain pycnidia develops in the culture.

2. If either the pulp of the subcortical tissue or the pulp of the medullary tissue (collected uncontaminated) of disease-free vine shoots taken from plants free of any disease

is cultured on sugar agar, the same form of *Sporothricum* is obtained in the cultures as that which grows spontaneously on the pycnidia or that is obtained in cultures inoculated with their contents.

The culture of this form of *Sporothricum* has a dirty olive-green color and subsequently becomes blackish-grey.

The pycnidia or sclerotia of Guignardia bildvellii are therefore abnormal formations of organic vine mold, and not formations of a heterogeneous parasite.

Conothyrium diplodiella (white rot of the vine) and *Septoria ampelina* (*melanosis*) are formations of organic vine mold that are confused with *Guignardia bildvellii.*

It follows from this study that, as in the case of the potato, vine diseases are not caused by parasites, but by unfavorable growing or weather conditions; the molds or various formations that characterize these diseases are only witnesses to them and not the initial cause; they are abnormal vegetative forms of organic vine mold.

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Since these diseases are not parasitic in nature, and molds such as downy mildew or *Botrytis, Oidium*, and *Guignardia bildvellii* are formations of organic mold, their treatment with anti-parasitic chemical products is therefore illogical. If certain chemical products have a favorable effect on some of these diseases, it is not by destroying the molds considered to be their initial cause, because this destruction would inevitably lead to that of the less resistant organic mold; they can only act by stimulating, on the contrary, the latter's vegetation.

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VI. Lettuce Disease

I have only studied the disease attributed to *Bremia lactucae*, the sucker. *Bremia lactucae* is not a parasite; it is the *Peronospora (or Botrytis cinerea)* form of lettuce mold.

To reproduce the mold that causes Miller's lettuce, simply place a few healthy lettuce leaves in a small, closed crystallizing dish; they rot and become covered with an abundant gray mold that is *Botrytis cinerea*, but which, in reality, is *Bremia lactucae*; indeed, suitable preparations of this mold show, alongside gonidiophores typical of *Botrytis cinerea* (Fig. 1, pl. 151), gonidiophores of various types, characteristic of *Bremia lactucae* (Figs. 2, 3, 4, 5, 6, pl. 151). It is also noted that gonidia form all along the branches (Fig. 7).

Subcultured on agar, *Bremia lactucae* shows spontaneous transformations that are common in organic molds of plants cultivated in vitro:

1. The spontaneous transformation into *Fusophragmium* (Fig. 10).

2. The transformation into *Verticilium* (A, fig. 9, pl. 151).

3. The transformation into a gonidial form not yet described (*Stylosporium*). The sterile spores, born along the branches, consist of a short, straight or slightly arched rod (B, B, fig. 9). I found this same gonidial form by cultivating on sweet carrot the spores of the ochre spots attributed to *Septoria lactuca*: in this case, there existed, in addition to the isolated spores along the branches, numerous groups of four spores gathered at the end of a short lateral branch like the sterigmates of a *Verticilium.*

4. The transformation into *Penicilium* form: filaments emanating directly from the mycelium of *Bremia lactucae* end in the characteristic fruiting bodies of *Penicilium*. This transformation has already been noted and illustrated for *Plasmopara viticola.*

There can be no doubt that these are indeed transformations, because the fruiting bodies that characterize them are observed on mycelial filaments emerging on the large branches of *Bremia lactucae.*

VII. Diseases Attributed to Uredinae

Uredinae are currently considered parasitic fungi characterized by specially shaped spores and a particular evolution. Their presence on plants is manifested by pustules or erosions disseminated on the leaves and stems. They affect a considerable number of plants and are thought to be the agents of a considerable number of serious diseases.

Here, we will only discuss a few types of *Uredinae,* taken as examples, in order to determine the nature of the diseases believed to be caused by them and their parasitism.

We will study in turn:

1. *Puccinia malvacearum,* forming pustules on the leaves of *Althoea officinalis.*
2. *Puccinia graminis* and *Puccinia triticina,* agents of wheat rust.
3. *Puccinia maïdis,* agent of corn rust.

Study of Puccinia malvacearum

Puccinia malvacearum forms a large number of brown hemispherical pustules containing spores on the underside of the leaves of *Althoea officinalis.* Note that these pustules do not form on young, growing individuals, but in autumn on individuals in the process of decline.

These pustules are not formed by a parasitic fungus of the order *Uredinae*, but by the organic mold of the *Althoea* in the process of decay.

The demonstration of this fact results from:

1. The existence, in the healthy and normal parenchyma of the *Althoea* leaf, of normal elements identical to those of the teliospores of a uredo.

2. The origin of the teliospores and their formation by the mycelial branches of the normal parenchyma of the leaf.

3. Study of the constitution of the normal parenchyma of the *Althoea* leaf.

4. On the formation, by culturing the spores of a uredo, of the gonidial forms of the organic mold of *Althoea.*

5. On the formation, by culturing the pulp of the central tissue of the healthy stem, of gonidial forms identical to those which develop by culturing the spores of a uredo.

We will successively study the constituent elements of the uredo, the constitution of the leaf parenchyma, and the gonidial forms obtained by culturing the spores of the uredo.

Constituent elements of a uredo and the leaf parenchyma.

The contents of a uredo being dissociated in a drop of water on a glass slide, fixed by rapid desiccation and then stained with dahlia, we first note that the shape of the teliospores (Fig. 1, 2, pl. 152) is far from constant; we do find some which have the elongated shape depicted by certain authors (A, B, fig. 152), but most have a different shape; instead of being bilocular, they can have three or four locules (D, fig. 1); others are unilocular (E, fig. 1, B, B', fig. 2). Others are extended at their end by a mycelial filament (A, A, fig. 2) or by a second spore (E, fig. 1); this last arrangement, which is that of *Alternaria*, is observed in most cultures of *Fusophragmium;* The elements described here are, in fact, fruiting bodies of the *Fusophragmium* type with one, two, three, or four loges, as will be seen later.

The analysis of the nature of these spores requires knowledge of the normal constitution of the parenchyma of plants and, in particular, of the leaf of the *Althoea* described previously. We have seen on this subject (P. 168 and pl. 136, 137):

1. That the cells of the parenchyma are not independent and that they are connected in a chain by a very short mycelial filament going from one to the other.

2. That a chain of cells or an isolated cell is never independent and is always formed by a mycelial filament starting from one of the main mycelial branches which, grouped in bundles, constitute the veins of the leaf.

On the other hand, it has been demonstrated that around every cell there is a supporting network or carcass formed by woody filaments.

If, recalling these facts, we compare the shapes of the teliospores in Figures 1, 2, 3, 4, Plate 152, with the shapes of certain cells of the parenchyma in Plates 136, 137, we find

that some of them are identical, for example: B, B', Figure 2, Plate 152 and J, M, Figure 5, Plate 136; H, I, Figure 1, Plate 152 and Z, Figure 5, Plate 136 and I, Figure 1, Plate 137.

If we dissociate a healthy fragment of *Althoea* leaf parenchyma with needles in a drop of water, we also find, in the dissociated elements, isolated elements of a shape identical to the teliospores (Fig. 7 and 8, pl. 153).

We also observe large isolated mycelial branches near their branched end, *identical to the gonidiophores of Botrytis;* these branches most often bear elements identical to the teliospores (A, fig. 1, A. B, fig. 3, pl. 153). The origin of the teliospores can be established by a fact even more precise than this identity of form with the normal elements; in fact, *they bear the mark of their origin in the supporting network which continues to form on their surface in the uredo, and which constitutes for them a decisive proof of identification.* The filament constituting this carcass is visible on most of the in the uredo, and which constitutes for them a proof of decisive identification. The filament constituting this carcass is visible on most of the teliospores; it is very apparent in plate 152, in H, figure 1, C, figure 3 and on all the teliospores of region F, figure 3.

Figures 3, 4, 5, 6, Plate 152, are photographs of a preparation obtained by dissociating the entirety of an uredo, including the underlying area. In Figure 3, a complete demonstration is given by the direct connection between a large mycelial branch of parenchyma A, branch B emanating from it, and the thin teliospore that terminates it, attached to teliospore E. This is formed by a filament emanating from the large mycelial trunk located further to the left. In Figure 4, examined under a magnifying glass, we will also see another teliospore formed at the end of a mycelial filament emanating from the large central branch of the figure.

These observations demonstrate:

1. That teliospores have the normal conformation of the elements of the plant parenchyma on which the uredo is formed, and that they possess, in particular, a supporting carcass.

2. That, in the normal and healthy parenchyma of the plant, there exist elements identical to all forms of teliospores and that these elements are born, like them, at the end of mycelial filaments emitted by the large branches constituting the veins.

Teliospores are therefore formations of organic mold constituting the normal parenchyma; they differ mainly from its elements by the loss of chlorophyll. In the underlying tissue of the uredo there is a network of mycelial filaments tangled in all directions (Fig. 6, plate 152); these filaments have been taken for the mycelium of the parasite. They are actually made up of organic mold and emanate from large fibrous mycelial branches arising from the veins; Figure 6, plate 152, shows one of these branches A and its division into fibrils. This underlying mycelium bears sessile spores of a particular form which will allow us to demonstrate its origin by another method.

Study of the gonidian forms developed by the in vitro culture of the spores of a uredo.

Cultivation of the teliospores of a uredo gives rise to the gonidian forms of the organic mold of *Althoea*.

To demonstrate this fact, the contents of a uredo, taken with a fine wet point, are inoculated onto sugar agar, after tearing its cap and dissociating its contents by scraping. After two to four days, hairy, olive-green colonies develop in the inoculated tubes. These colonies are covered, for the first few days, with fruiting bodies of the *Sporothricum* type shown in Figure 1, Plate 154. After a few days, a bluish-grey colony, which develops in the centre of each of the first, contains fruiting bodies of the *Fusophragmium* type (Figs. 3, 4, 6, Plate 154). This second type is an evolution of the first, which is demonstrated by the transformation which takes place in the centre of the primitive colonies; Moreover, by examining mycelial filaments taken from the intermediate zone in the blue-grey and olive-green regions, we observe, on the same filament, the two types of fruiting bodies (Fig. 2).

In well-developed cultures, we note that, among the fruiting bodies of *Fusophragmium*, there are a certain number which are bilocular and have exactly the shape of telentospores. A certain number of them are marked with a cross in figures 3, 4, 5, 6; we note, on the other hand, all the forms of elongated teliospores, tri- or quadri-locular, which the uredos contain.

These colonies therefore present the same gonidial type as the aredos, but their fruiting bodies evolve more completely to the Fusophragmium type.

We have noted above that, in the tissue which constitutes the deep or underlying zone of the uredo, there are tangled mycelial filaments bearing particular fruiting bodies; by examining them under a magnifying glass (Fig. 6, pl. 152), we see that they are of the *Sporothricum* type and identical to those which develop on the mycelium of the in vitro culture (Fig. 1, pl. 154); this mycelium also gives rise to teliospores identical to those of the uredo.

We see, therefore, that there is a perfect correspondence between the fruiting bodies obtained in cultures and those of a uredo.

This established, it is easily demonstrated, in the following way, that the fruiting bodies obtained are gonidial forms of the organic mold of the plant: take a large fragment of healthy *Althoea* stem. The bark is torn off in large fragments and the subcortical tissue remaining adherent to the stem is scraped with the blade of a scalpel, then the pulp obtained is spread on sugar agar. After three to six days, olive-green colonies are obtained, identical to those obtained previously by teliospore culture and containing the same fruiting bodies of the *Sporothricum* and *Fusophragmium* types.

The teliospores of the uredo of *Puccinia malvaccarum* are therefore cellular elements of the parenchyma of *Althoea*, *and their spontaneous development can be obtained by cultivating, in vitro, any part of the plant's parenchyma; they are therefore fruiting bodies of the organic mold specific to the plant.*

Consequently, uredo is not the manifestation of the presence of a parasite in the leaf, but the manifestation of the decay of the organic mold at certain points, under the influence of unfavorable causes; for *Althoea,* it appears that no other cause should be blamed than the natural decline of the plant, which, being an annual, always normally decays at the end of summer.

From these facts, it follows that Puccinia malvacearum does not exist and that the uredo attributed to its presence is an autogenous formation of the plant. The study of other species of *Puccinia* leads to the same result and demonstrates that fungi of the Uredinaceae family do not exist.

Study of wheat rust

Here, we will only discuss *Puccinia glumarum*, the agent of yellow rust. Indeed, we will see later that there is no difference in their origin between *Puccinia glumarum, Puccinia graminis, Tilletia,* and *Ustilago.*

We know that two forms of fruiting bodies develop on wheat leaves affected by rust: linear sori containing elongated, bilocular teliospores and uredos containing round uredospores. The teliospores emit, by germination, basidio-spores which could only develop on barberry where they would form an *oecidium;* the oecidiospores of this would be the source of infection of the wheat.

This cycle of development of wheat rust does not exist because it is made up of normal elements of the leaf parenchyma modified in their development and appearance, mainly by the loss of their chlorophyll.

The demonstration of this fact results from:

1. Examination of the constitution of the normal parenchyma of the wheat leaf, and the normal existence of urediniospores and teliospores in this parenchyma, of which they are the constituent elements.

2. The identity between the gonidial forms obtained by culturing the teliospores and urediniospores of *Puccinia glumarum*, and the gonidial forms of organic wheat mold.

Constitution of the parenchyma of the wheat leaf.

This study was carried out on young wheat leaves, germinated for eight days, and chosen from the best of the selected species. The composition of the parenchyma described here is therefore that of young, normal leaves, free from any defect or contamination. In young leaves, the parenchyma is made up of series of longitudinal and parallel cellular elements; each series is formed by two, three, or a greater number of mycelial tubes originating from the woody libero bundles, giving rise to a large number of filaments or lateral ramifications on which or at the ends of which the cellular elements of the parenchyma arise.

These cellular elements are membranous sacs, each containing a regular, spherical ball, carried at the end of a peduncle which is the formative mycelial branch and which emanates from the neighboring libero-ligneous bundle; the membranous sac which contains the ball is formed by a supporting carcass whose constituent fibers emanate from a spiral vessel of the libero-ligneous bundle; these fibers constitute the membrane of the sac by their spread on a single plane.

A certain number of these pedunculated balls are visible in figure 9, plate 155, at the points marked with a cross and in the region; by going over the figure with a magnifying glass, one will see others, several of which are equipped with their pedicle, all along the bundle which constitutes the black band located at the bottom. In figure 8

one sees a series of them in A, half torn from the parenchyma, and three teliospores in B, B, B.

I limit myself to these indications for the wheat leaf, because the composition of the parenchyma of the leaf of cereals, or of grasses in general, is studied further on in much more detail and shown with much more clarity in the photographs of the leaf of corn, which lends itself much better than the leaf of wheat to these observations.

The pedunculated spherical balls, arranged in longitudinal and parallel series along the libero-ligneous bundles, *are the same as those that fill the uredos, and which are called uredospores;* on the healthy plant, the spherical balls, normal cells, are formed by fine sinuous, *branched mycelial filaments, bearing green spores which are the chlorophyll grains*; in the uredos, the spherical balls, here called uredospores, have the same constitution, but the chlorophyll has disappeared; the fine interior spores have become yellowish or colorless.

As for the teliospores, they have the same origin; they are normal elements included among the others of spherical shape and having the same evolution as them.

This identification of urediniospores and teliospores with normal wheat leaf cells cannot be doubted, since they are established on young leaves, free from any defect or contamination.

Confirmation of this nature of uredospores and teliospores is also established by the characteristics of the gonidian forms to which they give rise when they are cultivated in vitro.

Culture of urediniospores and teliospores.

The urediniospore and teliospore sori are torn open on their surface with a point and the yellow dust they contain is inoculated onto sugar agar. The culture gives rise to colonies of hairy, white or grey molds.

A single colony can contain the three forms: Sporothricum (Fig. 1, pl. 155), *Fusarium* (Fig. 2), and *Sclerospora* (Figs. 3 and 4).

The mycelial filaments, which bear short, pointed spores of the Sporothricum type, also bear a few longer, bilocular spores, and all the intermediates between these two types.

In the *Fusarium* form, the bilocular spores (A, B, Fig. 2) appear to be homologous to the teliospores of the normal leaf parenchyma.

Some grey colonies possess both:

1. A *Fusarium* form, including some very long, thin, multilocular gonidia that appear identical to the ascospores of *Ophiolobus graminis* (Fig. 6, pl. 155).

2. *Sporothricum*-type spores (Fig. 6).

3. *Sclerospora*-type gonidia (Fig. 6).

4. Gonidia formed by a straight rod (B, Fig. 6) and identical to similar fruiting bodies that *Tilletia tritici* spores develop at the beginning of their germination (sporidia).

5. Gonidia of a particular shape (A, Fig. 6), identical to the so-called secondary sporidia that develop during the germination of *Tilletia tritici* spores.

Fusarium-type gonidia vary greatly in form; therefore, the distinction made between *Ophiolobus graminis* and *Leptosphæria herpotrichoïdes* (causes of foot rot in cereals) based on the different appearance of Fusarium-type ascospores is unfounded; these molds are the same vegetative form of organic wheat mold.

The *Sclerospora* form can develop on the mycelium of the *Fusarium* form, because on the same mycelial filament a *Sclerospora*-type gonidia can be observed alongside a *Fusarium gonidia* (Fig. 5): as in the other *Peronospora* forms of plants, *Bremia, Plasmopora,* etc., *Sclerospora*-form gonidia do not form only on specially shaped gonidiophores (Fig. 4); they also arise laterally along the entire path of the mycelial filaments (Fig. 3).

The molds obtained are gonidial forms of organic wheat mold; the demonstration of this fact results from:

1. From the identity between the *Penicilium* form (Pl. 216, 217), which is obtained by cultivating the pulp of young shoots of freshly germinated wheat, and the *Penicilium* form which appears in the culture of the pulp of the organs of a guinea pig injected with one of the gonidial forms just described.

2. From the identity between the gonidial forms obtained by in vitro culture of the uredospores or teliospores of *Puccinia glumarum*, with the gonidial forms obtained by in vitro culture of the spores of *Tilletia tritici* (wheat mold).

In summary, these observations demonstrate:

1. That the urediniospores and teliospores, considered to be the characteristic fruiting bodies of *Puccinia glumarum*, are normal elements of the wheat leaf parenchyma undergoing abnormal development, either under the influence of unfavorable cultural or meteorological conditions (persistent rains), or under the sole influence of aging (natural decline of annual plants).

2. That the parasitic fungi of wheat, and more generally of cereals, designated under the name *Puccinia*, do not exist.

3. That cereal rust is an autogenous disease characterized by a deformation of the organic mold of the plant, at certain points in distress, under the influence of the causes just indicated.

Thus, appears the accuracy of the observations that led Eriksson to believe that *the influence of contamination in the development of rusts has no importance that has been attributed to it*; wheat is not infected when it is more than 25 meters from barberry; *on the other hand, wheat rust exists just as well in regions where barberry does not exist* (Australia).

The fact that *Puccinia* do not exist, and that the fruiting bodies attributed to them *are normal elements of the wheat leaf*, demonstrates that the evolutionary cycle described for *Puccinia* does not exist any more than for them.

Eriksson's observations led him to admit that grasses possess in their tissues the germs of parasitic Uredineae. This hypothesis, while inaccurate since parasitism does not exist, is nevertheless correct relative to the very origin of the spores.

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The possibility of contamination, that is to say, the development on wheat leaves of spores that have been spread there, appears entirely natural, the leaf parenchyma being the most favorable culture medium for their development; *but the development of teleutospore sores or uredos on a healthy plant, experimentally contaminated, does not prove that it puts it in a state of notable inferiority.*

Indeed, what reduces the production of wheat affected by rust is not the presence of this rust, which is only a sign of the state of suffering or decay of the plant, but this state itself and the initial cause that provokes it. In a healthy plant, experimentally contaminated and not subject to the usual initial causes that develop spontaneous disease, it is probable that the development of rust is not accompanied by the decay of the plant.

The question of contamination is of little importance in a wheat field where all individuals are subject to the same causes of suffering and equally exposed to the development of rust.

Since the disease is caused by excess humidity and the origin of the rust is known, to avoid it, it is advisable to sow in a rainy region such as northern France, for example, not wheat accustomed to a dry climate, that is, from a country where rain is rare, but seeds accustomed to equal or even greater humidity.

It is likely that the state of suffering caused by persistent rains comes mainly from *the washing of the soil and the harmful effect it has on the specific mycelial and bacterial culture that grows around the roots of the plant.* The elements of this culture can be carried away by the washing of the soil; this can, moreover, slow the multiplication of zymospores and other active bacterial elements by the cooling it causes.

One way to combat the harmful effects of excess moisture therefore appears to be to promote the development of inter-root mycelial culture by providing it with the nutrients (fertilizers) best suited to its development.

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VIII. Study of Wheat Decay.

Wheat decay is attributed to a parasitic fungus of the *Tilletiaceae* tribe *(Basidiomycetes): Tilletia tritici.* This parasitism does not exist; it is the normal cells of the grain, deviated in their evolution by a state of suffering of the plant, that have been taken for the spores of Tilletia; proof of this fact is provided:

1. By the direct examination of the spores of a decayed grain.

2. By the identity between the gonidial forms developed in in vitro cultures of the spores of *Tilletia tritici* and the gonidial forms of organic wheat mold.

Direct examination of the spores of Tilletia tritici.

For this examination, a grain of decayed wheat is opened and part of its contents is placed in a drop of water on a glass slide. It is spread out, dried while hot, and lightly stained with dahlia. The following characteristics indicate that the blackish granules contained in the grain are parenchyma cells and not parasite spores:

1. The cells do not all have the spherical shape attributed to *Tilletia* spores; they are often ovoid in shape, and *a large number still bear vestiges of the supporting framework.*

2. The cells (spores) remain assembled in groups of five to ten elements joined in a chain by a very short mycelial filament (A, fig. 1, pl. 156), *the normal arrangement of the parenchyma elements of a healthy grain.*

Certain cells (C, C, fig. 1) emit filaments bearing rounded bodies which are young cells developing on the large ones.

In B, figure 1, we see a fragment of mycelial branch of the organic mold giving rise to one of the cells considered to be *Tilletia* spores (normal arrangement of healthy grain).

The persistence of the vestiges of the supporting framework on the surface of the *Tilletia* spores and the existence of the mycelial filament which formed them and joined

them in a chain, constitute decisive elements of identification with the normal cells of the grain parenchyma.

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Spore culture of Tilletia tritici.

The blackish-brown dust contained in these carious grains is spread on sugar agar (5% maltose, 5% glucose), and from the third to the fifth day, the cultures produce either short-haired, greenish-grey colonies, each of which contains fruiting bodies of four or five different types, or short-haired, green colonies of the pure *Penicilium* type.

In the greenish-grey colonies, the following fruiting bodies are observed (Pl. 156):

1. Sporidia, rods formed by the segmentation of short filaments that arise on the mycelial branches (A, fig. 2, pl. 156). These filaments are homologous to the para- physes of *Ophiolobus graminis* and *Leptosphæria herpotrichoïdes.*

At certain points, the filaments that segment into sporidia appear in groups of four or five.

2. Bilocular *Fusarium gonidia* and some (rare) three- or four-celled (Fig. 2). Comparing this *Fusarium* form to that obtained by culturing wheat leaf rust elements, it is easy to see that they are identical, but unequally developed, the former having remained rudimentary.

Some of the gonidia are identical to the teliospores of wheat rust (A, A, Fig. 2).

3. *Fusophragmium*-type fruiting bodies (A, Fig. 3) identical to those that develop in cultures of rust teliospores.

4. Short and massive formations (B, fig.3, fig. 4, fig. 5; A, fig. 6) appear to be pear-shaped asci, emitting short bilocular *Fusarium gonidia*; these asci open by expulsion of the cap, the lower edge of which is visible in figures 4 and 5.

5. Elongated asci arising laterally on the mycelium branches (B, fig. 6; C, D, fig. 7) and emitting small bilocular gonidia and fine filaments (paraphyses) which segment into short sporidia (B, fig. 7). The two gonidia A, which are placed at the end of ascus C, appear to arise on its outer wall by a short pedicle, like the gonidia located at the lower part of ascus D. Ascus D, almost empty, emits gonidia and filaments at its end.

The green colonies contain an identical *Penicilium* form (Fig. 8, 9, 10, pl. 156; Fig.1 to 7, pl. 216; fig. 1, 3, 4, 5, 6, 10, 11, 14, pl. 217) to that of organic wheat mold; this is easy to

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obtain by the following process: wheat grains are sown in a pot containing sterilized sand and kept under a bell jar to protect from dust. They are watered with sterile

water; when the young leaves have reached ten to twelve centimeters in height, they are cut, taking care not to soil them, they are pounded in a sterile mortar, then the pulp thus obtained is spread on sugar agar; after three or four days, small green colonies of the *Penicilium* form of organic wheat mold are still developing in one or two tubes out of six sown (Fig. 8, 9, 10, pl. 216). This form is also obtained by culturing the pulp of the organs of a guinea pig inoculated with the pulp of young leaves (Fig. 2, 7, 8, 9, 12, 13, 15, 16, 17, pl. 217).

From the examination of these different gonidian forms, it appears that the spores of *Tilletia tritici*, inoculated on agar, give rise to:

1. To the *Fusarium* and *Fusophragmium* forms as well as to sporidia identical to those which are born in cultures of wheat rust elements.

2. To terminal fruiting bodies in the form of globular asci emitting both *Fusarium*-type gonidia and sporidia.

3. Has elongated asci arising along the mycelial branches and similar to those of *Ophiolobus graminis* and *Leptosphæria herpotrichoïdes*.

4. Has the *Penicilium* form of organic wheat mold.

By culturing the normal elements of young wheat leaves, we obtain not only the *Penicilium* form, but also the *Fusarium, Fusophragmium, Alternaria, Sporothricum,* and *Sclerospora* forms, identical to those contained in plates 155 and 156; on the mycelium of the *Sclerospora, Fusophragmium,* and *Alternaria* forms, we observe the presence of straight sporidia and so-called secondary, arched sporidia, identical to those which are born on the germinative filaments of the spores of *Tilletia tritici*; we also obtain a white mold without a characterized gonidial type, but giving rise to rounded or pear-shaped black peritiecia, similar to those of *Ophiolobus graminis* and *Leptosphæria herpothricoides.*

From these facts, we must conclude:

1. That *Tilletia tritici* does not exist; what has been taken for the spores of a parasitic fungus are the cells of the grain parenchyma whose evolution is modified as a result of the plant's state of suffering.

2. That the cause of wheat bunt lies not in infection by a parasitic fungus, but in defective cultural or meteorological conditions.

3. That no antiparasitic treatment can therefore be useful and that only the choice of cultivated varieties and the improvement of cultural conditions can have a favorable effect on the disease.

Study of rust and smut of corn.

Corn rust is attributed to a parasitic fungus, *Puccinia maydis* (Uredinaceae), and smut to another fungus of a different tribe, *Ustilago maydis* (Ustilaginaceae).

The following observations show that these two parasites do not exist and that the elements taken for their mycelium and spores are the normal elements of the plant parenchyma deviated in their form and evolution. The evidence for this fact is provided:

1. By a study of the normal parenchyma of the healthy corn leaf, demonstrating that urediniospores and teliospores are the normal elements of the leaf parenchyma.

2. By a study of the contents of the sori, urediniospores, molds growing on the surface of the leaves, and smut tumors, demonstrating that smut spores are formations of the organic mold of corn.

3. By the culture of the urediniospores and teliospores of *Puccinia maydis* and the spores of *Ustilago maydis*, followed by a comparison of the molds obtained with the gonidial forms of the organic mold of corn.

Let us examine each of these points in turn:

Study of the parenchyma of the healthy corn leaf.

Selected corn grains of one of the best species are sown in moist, sterilized sand, contained in a sterile pot placed under a sterile bell jar. The young leaves are examined when the shoot reaches 12 to 15 centimeters in height. The epidermis of a leaf is removed over the largest possible surface area and placed in two or three drops of water on a glass slide, the flaps of epidermis and the underlying part; it is dried quickly on the

heating plate, then mounted with Canada balsam under a coverslip, without having colored or after moderate coloring by the dahlia, followed by a new drying.

Figures 1 and 3 of Plate 157 are two photographs of a flap of epidermis to which part of the underlying parenchyma has remained adherent. Below the transparent epidermis, we see the rows of parallel spherical cells that constitute the parenchyma of the healthy leaf or, in a leaf affected by rust, the urediniospores of the uredinios. Some of these cells have their formative pedicles visible. On the edge of Figures 1 and 3, in A, where the epidermis is missing, we see two rows of naked cells, some of which have their formative pedicles visible. Two or three elongated elements, which are teliospores, are also visible by transparency in B and C, for example (Fig. 3). In D, we see, in the deep zone, a fairly large mycelial branch running from which the ramifications that form the spherical cells originate.

Figure 2 represents two small libero-ligneous bundles containing two spiral vessels and their anastomosis; on each side of the bundles, we see the spherical cells arranged in rows, A, B, C, D, E, F, G, H; between these elements are lodged quite numerous cells called teliospores, but which rarely appear in such a way as to be visible on a single plane; in figure 2 we see one, C, of great clarity, lodged between two spherical cells; four others less clear in L, M, N, O and others in P. The eye which runs over a preparation easily discovers a large number of them.

We also observe in the preparations a large number of oval-shaped elements, pointed at their ends, identical to the spores of the *Sporothricum* form that we obtain by cultivating the pulp of the tissues of most plants on agar (A, B, C, D, fig. 4, pl. 157). These are the young elements that will become the teliospore-shaped cells.

Figures 2 and 4 provide an important indication of the structure and role of the spiral vessels; they are surrounded by a membrane or sheath, independent of the spiral wall; the sheath F of the spiral vessel E (Fig. 4) is separated from the vessel, at F and G, by a bundle of mycelial fibers originating from the spiral fiber and which emerge from the sheath at this point; these fibers, emanating from the spiral vessels, are those which will constitute the supporting carcass of the parenchyma elements; this fact has already been noted previously.

It follows from these observations that the normal parenchyma of the corn leaf is made up of spherical or spindle-shaped elements identical to the urediniospores and teliospores of rust; the identification is completed if we compare the shape of the normal elements of the parenchyma (Fig. 1, 2, 3, 4, pl. 157) with the teliospores and urediniospores removed from sori or uredos of corn rust (Fig. 1, 2, 3, 4, 5, pl. 158).

This comparison leads to the observation that the shape, size, and composition of the elements are the same.

Examination of the elements contained in the sori and uredos of corn rust.

In descriptions or studies of corn rust, it is stated that the teliospores of *Puccinia maydis* are contained in elongated sori, while the urediniospores are contained in uredos.

Indeed, some sori are found which contain almost exclusively teliospores and with which only rare urediniospores are mixed (Figs. 1 and 2, pl. 158). Similarly, some uredi-niospores contain almost exclusively urediniospores (Figs. 3, 4). But other sori or urediniospores contain both teliospores and urediniospores (Fig. 5). The explanation

for this fact is provided by the study of the constitution of the parenchyma of the healthy corn leaf on which these two elements normally exist mixed.

Many uredos are formed by the vegetation of the *Sporothricum* form of corn (Figs. 6, 7, 8). The mycelial filaments bear, in addition to the sessile oval spores, generally bilocular, round spores identical to those which form in the charcoal vegetation which covers the leaves and glumes or which constitutes the tumors.

The uredos containing this gonidial form are, at first, small isolated brown or black dots. Later, the internal vegetation of the uredo forms a small brown halo around the orifice, which gradually spreads and comes to constitute a spot of one centimeter or more. The mycelium spread over the leaf and which constitutes this spot has the same constitution as that of the uredo (Fig. 9, 10, pl. 158). The mycelial filaments which constitute this vegetation are identical to those which form the teliospores (Fig. 1, 2); these filaments bear either round spores similar to the spores of anthrax and which, as they grow, become similar to uredospores (Fig. 7, 8, 9, pl. 158), or oval spores which are generally bilocular.

If we now examine the uredospores (Fig. 3, pl. 158) and compare them with the normal spherical elements of the leaf parenchyma (A, figs. 1 and 3, pl. 157), we see their identity. Examined very closely, with a magnifying glass, these elements have the same constitution, the same shape and the same dimensions. The pedicle forming the uredospores is often, like the pedicle forming the normal parenchyma cells, surrounded by a spiral filament. Like normal parenchyma cells, the uredospores emit short mycelial filaments at the end of which a new cell is formed (A, fig. 5, pl. 158). The same fact exists for the teliospores. The identity is therefore complete between the urediniospores, the teliospores, and the normal elements of the leaf parenchyma.

The same identity is observed when comparing the teliospores of the rust sori (Fig. 1, 2, pl. 158) with the normal teliospores of the healthy parenchyma of the young maize leaf (Fig. 2, pl. 157). Examination of the teliospores of the rust spores under a magnifying glass reveals the filament constituting the supporting carcass and the coiled and branched myeloid filament constituting the interior of the element.

Teliospores and urediniospores are therefore normal elements of the leaf parenchyma, but they have lost their chlorophyll because their development is modified by nutritional or unfavorable climatic conditions. Corn rust is therefore not caused by a heterogeneous parasitic fungus.

On the other hand, *Sporothricum*-type vegetation, which develops in the urediniospores, spreads outside and *is the starting point for the charcoal growth on the leaves and glumes (sludge).*

Direct examination of the spores taken from anthrax tumors shows that they are cells identical to urediniospores, but smaller; they are, in fact, linked together in a chain; the mycelial filament that unites them and forms them can be distinguished between them; some are equipped with a fairly long formative filament around which a spiral filament can be distinguished. The arrangement and mode of formation of these cells is therefore identical to that described for the spores of wheat rot and for the spores contained in anthrax-prone barley and oat grains.

Corn smut, therefore, does not differ from rust; it only constitutes another aspect of the same disease, rust being constituted by an abnormal vegetation of the normal *elements in the parenchyma, and smut by vegetation on the leaves, outside the plant, abnormal mycelial elements of organic mold that emerge and proliferate outside the uredos.*

This proliferation, which becomes enormous in the axils of the leaves or glumes, constitutes smut tumors.

The culture of smut spores confirms this conclusion; it gives rise to the gonidian forms of organic mold of corn.

Culture of the teleutospores and uredospores of Puccinia maydis and culture of the corn smut spores.

The teliospores and uredospores cultivated on agar give rise to a *Sporothricum* form (Fig. 8, pl. 159) and a *Mucor corymbifer* form identical to those of organic mold of corn (Fig. 12, 13, pl. 159).

A mass of spores taken from the center of a corn smut tumor, soaked in sterile water and placed in a closed crystallizing dish, germinates rapidly and gives rise, on its surface, to a mold that presents the following gonidial types:

1. Certain germinative filaments bear large, elongated, oval, bilocular gonidia (Fig. 1, 2, pl. 159). These gonidia are formed at the end of the germinative filament emitted by the smut spore or at the end of a short lateral branch of this filament (Fig. 1 and 4, pl. 159). *They are found on mycelial filaments of the Mucor type that are obtained by cultivating the healthy tissues of the young corn leaf (young shoot of 10 centimeters)*. Figures 4 and 5 represent these spores in the process of formation.

2. Other germinative filaments develop in a monosterigmatized form, giving rise either to fine, round spores (Fig. 3) or to large gonidia which appear to be formed by the agglomeration of spores formed at the end of the filament (*Cephalosporium* type) (Fig. 5); certain filaments which do not bear sterigmates form laterally a multitude of fine, sessile, oval spores (Fig. 5).

3. Certain elements, probably of secondary formation, agglomerate to form germinative masses emitting mycelial filaments bearing fruiting bodies of the *Penicilium* type (Fig. 6, 7, pl. 159).

The mold that gives rise to the large oval spores spontaneously transforms into the *Mucor* type, and the latter into *Fusarium* or *Penicilium.*

The three forms *Mucor, Fusarium,* and *Penicilium*, thus obtained, are very characteristic: their comparison with the gonidial forms of organic mold in corn makes it easy to identify them and to conclude that the spores of corn smut are indeed elements formed by the organic mold of this plant.

Note, moreover, that the leaves and glumes of diseased plants take on, at certain points, either a wine-red color or an orange color. By cultivating healthy corn tissue, sterile molds or molds without a characteristic gonidial type are obtained, which take on either the intense wine-red color or the bright orange color.

Gonidial forms of organic mold in corn.

The gonidial forms of organic mold in corn are easily obtained:

A. By culturing on agar the pulp of the medullary tissue or subcortical tissue of a healthy corn stalk, taken without contamination. On the cultures, the following gonidian forms appear:

1. The *Sporothricum* form (Fig. 8, pl. 159); the mycelial filaments bear oval spores and round spores.

2. The *Penicilium* form (Fig. 9, 10, 11, pl. 159 and figs. 1 to 18, pl. 226).

3. The *Mucor* form (Fig. 12, 13, p. 159). This form is of the corymbifer type. It is difficult to preserve it in a pure state. It gives rise, by spontaneous transformation, to either the *Penicilium* form or the *Fusarium* form (Fig. 14, 15, pl. 159).

This *Fusarium* form is very distinct; its mycelial filaments bear fine ramifications ending in small oval spores identical to those that arise on the mycelium of the *Monosterigmata* form developed by anthrax spores (Fig. 5, pl. 159).

In this transformation, the first mycelial filaments of *Mucor* or *Fusarium* forms arise directly on the mycelial branches of *Mucor.*

B. By inoculating the guinea pig with an emulsion of subcortical tissue pulp from a healthy corn stalk or crushed corn kernel, and culturing the pulp of its organs on agar.

The following conclusions can therefore be drawn from the observed facts:

1. The normal parenchyma of the healthy corn leaf is made up of elements identical to the urediniospores and teliospores of *Puccinia maydis*.

2. The sori or uredos of rust contain both urediniospores and teliospores with vegetation in the *Sporothricum* form of the organic mold of the plant.

3. This *Sporothricum* vegetation of the sori and uredos develops smut spores and, by spreading over the leaves and glumes, it constitutes the smut spots.

4. The smut spores of tumors are identical to the urediniospores and are normal spherical cells of the parenchyma modified in their development.

5. The urediniospores and teliospores of *Puccinia*, as well as the smut spores, give rise, by in vitro culture, to molds with gonidial forms identical to those of the organic mold of corn smut.

6. Consequently, the elements constituting *Puccinia maydis* and *Ustilago maydis* are not those of heterogeneous parasitic fungi, as these do not exist; the elements taken for their spores are normal elements of the plant parenchyma whose evolution is deviated as a result of the plant's suffering state. Corn smut and rust are two different aspects of the same disease.

7. There is therefore no real parasitism; the disease is caused by a defective adaptation of the plant to the climate, if it is of foreign origin, or by unfavorable cultural or meteorological conditions.

The disease can also be due to the normal decay that occurs with the aging of annual plants; a plant of foreign origin, accustomed to rapid growth in a hot, dry country, may reach extinction before its production is ripe. In choosing cultivated species, the normal duration of growth of the plant in its country of origin and the weather conditions to which it is accustomed must be taken into account.

Oat smut.

Oat smut is attributed to a parasitic fungus, *Ustilago aveni*. It develops not only on the glumes, but also in the grain.

The elements taken for the spores of *Ustilago aveni* are, as in the diseases previously studied, the normal elements of the ear (glumes or grain) deviated in their develop-

ment by a state of suffering of the plant due to defective cultural conditions or unfavorable weather conditions.
Oat smut is therefore an autogenous disease of the plant, not a parasitic one; *Ustilago aveni* does not exist.

The evidence for this is provided:

1. By direct examination of the smutty elements of the grains and glumes

2. By the identity of the gonidial forms, developed in cultures of smut spores on agar, with those of the normal organic mold of oats obtained by culturing the pulp of young healthy leaves.

Examination of the smut elements of the grains and glumes.

The smut elements from the grains do not have the same form and the same mode of evolution as the elements from the glumes.

In the grain, the carbonaceous elements have the same constitution as those of wheat smut. They are normal cells of the grain parenchyma, deviated in their form and development; like normal cells of the grain parenchyma, they are connected to each other by a short mycelial filament; as in all parenchyma, the same cell can be connected to two or three different cells to which it gives rise; this arrangement, as well as the filaments connecting the cells together, are very apparent in Figures 45, Plate 160.

Examined at higher magnification, these cells have the same constitution as normal parenchyma cells; their color (become brown), their dimensions, their form, and their functions have changed because they have acquired a life independent of that of organic mold; they no longer form starch.

On the glumes, the shape of the carbonaceous elements is different. Figure 1, pl. 160, is a photograph of fragments of a dissociated glume with needles and stained with dahlia. We see the carbonaceous spores and, in A, B, the gonidial form that gives rise to them; this is the *Monosterigmata* form.

Gonidial forms developed in cultures of oat smut spores, or by culture of the organs of a guinea pig inoculated with these spores.

The culture of oat smut spores on sugar agar gives rise to:

1. The gonidial form *Sporothricum*.
2. The *Penicilium* form.

Culture on sugar agar of the pulp of the organs of a guinea pig (lungs, liver, spleen) inoculated with anthrax spores gives rise to:

1. The same gonidial form (*Monosterigmata*) (Fig. 2, 3, plate 160) as that which constitutes the anthrax growing on the glumes and which has just been reported.

2. A *Sporothricum* form.
3. A *Penicilium* form.
4. A gonidial form of *Aspergillus* of the *fumigatus* type (Fig. 6, plate 160).

5. A *Mucor* form not shown in the plate. But it is shown in figures 1 to 4, plate 228, relating to exanthematic typhus. The agent of this disease is in fact the organic mold of oats.

Gonidial forms obtained by culturing the pulp of young, healthy oat leaves on agar.

In these cultures, only the *Penicilium* form developed; it is identical to that produced by direct culture of anthrax spores and by culture of the organ pulp of guinea pigs inoculated with the spores. Culture of the organ pulp of guinea pigs inoculated with the pulp of young, healthy oat leaves gives rise to:

1. A *Penicilium* form (Fig. 1 to 7, pl. 229).

2. A *Monosterigmata* form.

3. A *Mucor* form.

These forms are identical to those obtained by culturing the organs of guinea pigs inoculated with smut spores. These facts therefore demonstrate:

1. That the elements constituting the smut of the grain or glumes of oats are elements of the specific organic mold of the plant, modified in their form and evolution.

2. That the spores of oat smut give rise to the normal gonidian forms of the organic mold of the plant.

3. That *Ustilago aveni* does not exist, since the elements that constitute it are formed by the organic mold of the plant.

4. That, consequently, oat smut is not a parasitic disease, but an autogenous disease whose cause must be attributed to defective cultural conditions or unfavorable meteorological conditions.

Barley smut

Barley smut is attributed to a parasitic fungus, *Ustilago hordei.* This parasite does not exist. It is the normal elements of the grain parenchyma, modified in their form and evolution, that have been taken for the spores of *Ustilago hordei.*

This fact is demonstrated:

1. By direct examination of the smutty elements of the grains and glumes.

2. By the identity of the gonidial forms obtained by culturing smut spores on agar or by culturing the pulp of the organs of the guinea pig, inoculated with these spores, with the gonidial forms of the organic mold of barley.

Examination of the smutty elements of the grains and glumes.

The carbonaceous grains contain large round granules which are parenchyma cells modified in their evolution. They have retained certain characteristics of these cells, notably the short mycelial filaments which unite them and vestiges of the supporting carcass; they are arranged in rows of four, five or six cells and some of them emit two or three mycelial filaments which are the origin of new series of cells.

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The round cells of the smut grain are much larger than the smut spores formed by the mycelium growing on glumes; they are, like the smut cells of the oat grain, homologous to the spores of the curried wheat grain (*Tilletia tritici*).

On the glumes, the smut spores are carried by long mycelial filaments; they are born either directly on these filaments or at the end of sterigmata that emit them in chains. Figure 1, Plate 161, shows the smut mycelium climbing along the hairs of the glumes. By examining a certain number of these hairs and the surface of the glumes, we can distinguish a few sterigmata that give rise to the chains of spores; Figure 2, Plate 161, shows them on the surface of a glume.

Identity of the gonidial forms of organic mold of barley and the gonidial forms obtained by culturing smut spores.

By culturing the smut spores of the glumes or the smut granules of the grain on agar, we obtain:

1. *Mucor hordei* (Fig. 8, 9, pl. 161).

2. The *Sporothricum* form (Fig. 5, pl. 161).

3. The perfect *Penicilium* form.

The *Mucor* form has an interesting peculiarity; the mycelium bears isolated sterigmata, or short branches ending in a group of sterigmata emitting chains of spores identical to those of anthrax.

Thus, the gonidian form *Mucor* can emit directly onto its mycelium either *Penicilium gonidiophores,* or *Fusarium gonidia*, or isolated sterigmata (*Monosterigmata* form).

By culturing on agar the pulp of the organs of a guinea pig inoculated with spores of *Ustilago hordei*, we obtain, in addition to the *Sporothricum* and *Mucor* forms:

1. A brownish-ochre mold with the same gonidial form as the smut growing on the glumes (Fig. 3, 4, pl. 161).

2. The perfect *Penicilium* form (Fig. 10, 11, pl. 161; figs. 2, 7, 9, 10, 11, 12, 13, pl. 218).

3. A rudimentary *Penicilium* form (Fig. 6, 7, pl. 161).

By direct culture on agar of the pulp of young barley leaves, we obtains the *Penicilium* form (Fig. 12, 18, pl. 161; fig. 1, 3, 4, 5, 6, 8, pl. 218). The *Monosterigmata* form is obtained quite easily, in addition to the previous ones, by culture on agar of the pulp of the organs of a guinea pig inoculated with a little pulp of young leaves or with the substance of a grain of barley crushed in water.

The two forms *Penicilium* and *Monosterigmata* of the organic mold of barley being identical to those obtained by culture of the spores of anthrax, it follows that these are indeed a modified form of the organic mold specific to the plant.

Ustilago hordei therefore does not exist, since the elements that constitute it come from the organic mold of the plant. Barley smut is therefore not a parasitic disease; it is a spontaneous plant disease due to unfavorable cultural or weather conditions.

As with corn smut, the identification between smut and organic mold of barley can also be achieved by comparing bacterial cultures. The bacterial form obtained by broth culture of young, healthy, crushed barley leaves is identical to that obtained by culturing smut. Let us add here that these cultures are those of the diphtheria bacillus; the pathogen of diphtheria is organic mold of barley.

Brown rot of fruits

Fruit rot is attributed to related parasitic fungi classified in the genus *Monilia*. This fungus develops on fruit in the form of small, yellowish or grey hemispherical projections, often arranged in circles. These projections are formed by the growth of a mold of the *Monilia* form. The mold forms sclerotia that remain inert for a year and later germinate, giving rise to a peziza.

I studied *Stromatinia fructigena*, which develops on apples and pears, and *Stromatinia cinerea*, which develops on plums.

Stromatinia fructigena.

This fungus develops on fruit, apples and pears, even adherent to the tree and on fruit that has fallen to the ground. Observing its development shows:

1. That it only ever appears on an area of the fruit that has previously been mortified and turned brown, that is, rotten.

2. That it appears, not on some of the fruit that has fallen to the ground, but on all of it.

3. That it appears just as well on healthy fruit, picked with care, carefully washed and sanitized, and kept under a sterile bell jar after bruising its surface to determine its mortification.

The fact that the fungus develops just as well on healthy fruit free from any stain already indicates to us that it cannot be a heterogeneous parasite. We have further proof of this in the following facts:

The mold that constitutes the hemispherical projections developed on fruits (*Monilia fructigena*, Bon.) has been classified in the genus *Monilia.* Upon examining this mold, it is difficult to understand why it was classified in this genus, since it has the appearances of both the genus *Cladosporium* and even those of the genus *Sporothricum*. In truth, it should rather be classified as a *Sporothricum* form.

Upon examining the small creamy-white, then yellowish, nipples that develop on the mortified regions of fruits that have fallen to the ground, we see that some of them turn green and are then constituted by a *Penicilium* form. If the mold constituting the yellowish nipples is cultivated on sugar agar, we obtain the development of a *Sporothricum* form that is clearer and more distinct than that which develops in the nipples, and in some tubes:

1. Either the direct development of the *Penicilium* form.

2. Either the development of the *Penicilium* form, in the center or at the periphery of *Sporothricum* colonies. This transformation of *Sporothricum* into *Penicilium* can be observed on a pure colony after several subcultures on agar.

The yellowish colonies of *Sporothricum* and the green colonies of *Penicilium* that grow on the dead regions of the fruit are therefore two different forms of the same mold. It is easy to prove that, if they have been collected from an apple or a pear, they are two forms of the organic mold of the apple or pear tree; a healthy apple is divided into two parts without soiling the section surface; this surface is scraped with the blade of a

sterile scalpel to obtain a fine pulp that spread on sugar agar. After a few days, one always obtains the development of one or two colonies of Penicilium identical to that which develops directly on the fruit; one can also obtain the *Sporothricum* form.

If, instead of the pulp of a fruit, one cultivates the pulp of the subcortical tissue of a healthy branch, one easily obtains the *Sporothricum* form and the *Penicilium* form.

The molds thus obtained by culturing the healthy tissues of the plant being identical to those which constitute the yellowish or green nipples developed on the mortified regions of the fruits, it follows that these are not heterogeneous parasites; they are gonidian forms of the organic mold of the plant.

Monilia fructigena therefore does not exist, and brown rot is not a fruit disease; the fungus that develops on a dead, brown area is a form of organic apple mold; fruit that has fallen to the ground, and sometimes fruit still bearing the tree, turns brown and rots because this is their natural progression and not because they are contaminated by a parasitic fungus; all fruit, regardless of the precautions taken to preserve it, ends up rotting like fruit that has fallen to the ground. When rot is early and rapid, the cause must be sought not in a parasite, but in trauma (bruising, insect bite) or in unfavorable climatic conditions (persistent rain, frost, etc.).

The molds that develop on apples, pears, or other fruits are not identical; they are different for each species, like the organic mold from which they derive. The *Stromatinia* of the apple is different from that of the pear.

The *Pezize* form, which arises from the sclerotia formed by the organic mold of the rotting fruit, is therefore a gonidian form of this mold; the interest of this form lies above all in the fact that, arising from the organic mold of the plant, *returned to its ancestral form,* it already constitutes a first stage in the mold's return to the organic form.

Stromatinia cinerea.

It is to this fungus that the rot of plums, peaches, cherries, etc. has been attributed. The fungus also develops on the twigs. It forms small gray nipples on the dead areas of the fruit.

This fungus, like the previous one, is not a heterogeneous parasite; it is a form of organic fruit mold. Gray nipples are formed by a mold of the *Sporothricum* form (*Monilia cinerea*). Some nipples develop in the *Penicilium* form. By cultivating the *Sporothricum* form on sugar agar, we obtain its transformation into the same *Penicilium* form as that which develops spontaneously on the fruits.

By cultivating the pulp from the center of healthy fruits or the pulp of the subcortical tissue of branches on agar, we easily obtain the *Penicilium* and *Sporothricum* forms identical to those which develop spontaneously on the fruits or on the twigs.

As in the previous case, brown rot is therefore not due to a heterogeneous parasitic fungus; the gray nipples which develop on the mortified areas are formed by a vegetative form of organic mold.

As for the mortification itself, which always precedes the development of the grey nipples, it is a phenomenon of the natural evolution of the fruit. This natural evolution is rapid when the fruit has fallen to the ground and exposed to humidity; it is even faster when the fruit is preserved under a humid bell jar.

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IX. Tree canker. Red rot disease. Nectria.

Nectria are considered parasitic fungi of trees; they appear as small pink or bright orange-red projections that lift and burst the epidermis. It is thought that the mycelium of the fungus grows in the affected branches and causes them to desiccate. It is generally accepted that canker in fruit trees always begins with the growth of *Nectria* in the bark.

These ideas are erroneous, as *Nectria* is not a parasite; it is a vegetative form of organic mold on the tree. *Nectria* does not dry out a branch; it only grows on branches in the process of dying, and it grows there precisely because they are in this state. Organic mold in pain changes shape and forms the formations called *Nectria*, but it never forms them if it is not previously in a state of dying.

A pustule of *Nectria cinnabarina*, dissociated and examined under high magnification, is shown to consist of the vegetation of a mold of the *Sporothricum* form; this mold, cultivated on agar, can be transformed into the *Penicilium* form. If the aseptic pulp of the subcortical tissue of a healthy branch taken from an unaffected subject is also cultivated on agar, these two identical gonidial forms are obtained.

The mold that constitutes the small red protrusions of *Nectria cinnabarina* is therefore a vegetative form of plant organ mold. If you cultivate plum *Nectria cinnabarina* on agar, you obtain the gonidial forms of plum organ mold, not that of another tree.

By cultivating *Sophora Nectria cinnabarina*, I obtained the gonidial forms of *Sophora* organ mold, forms that are completely different from those of the plum tree, although the *Nectria cinnabarina* of the latter species appears identical to plum *Nectria*. For example, by cultivating *Sophora Nectria cinnabarina,* you obtain a very particular *Mucor* form specific to sophora organ mold.

Thus, *Nectria* is not a parasite of trees and exists neither as a genus nor as a species.

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When the organic mold of a tree is suffering in a branch, *it loses its organic type and takes on an abnormal and independent vegetative form;* it can undergo multiple transformations as seen in the plant diseases previously studied.

Nectria, which has no specific existence and is only an appearance of the organic mold of the plant, cannot therefore be the cause of tree canker; it is, like canker itself, a manifestation of the state of suffering; the cause of canker lies solely in unfavorable cultural and meteorological conditions; its nature is, in short, identical to that of animal cancer; when the mold that constitutes canker is formed, it lives and spreads at the expense of neighboring healthy tissues, because its vegetation is independent and no longer obeys the laws of tissue development. As with cancer, there is only one way to prevent its spread: the total removal of the affected area.

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CHAPTER VIII

NATURE AND ETIOLOGY OF HUMAN AND ANIMAL DISEASES

Isolation of pathogens of contagious diseases

Original source of viruses

Nature and etiology of human and animal diseases.

Since an animal is made up of an organized mold, the diseases that affect it have the following causes, apart from parasitism by animals (helminths, insects, etc.):

1. An alteration of the vegetative activity or an abnormal deviation of the vegetative form of the mold constituting the organism.

2. Contamination of the organism by a heterogeneous mold.

This distinction is necessary, as organic mold can give rise to abnormal bacterial cultures or mycoses just as much as heterogeneous infective molds.

Diseases caused by an alteration of the vegetative activity or an abnormal deviation of the vegetative form of organic mold.

The immediate causes of these diseases are conditions unfavorable to the development of specific organic mold: cold, excessive heat, insufficient nutrients (poverty), excessive work (overwork), the harmful action of certain toxic chemical substances, among which alcohol must be classified (alcoholism); an excess of food, especially of certain foods (meat), creates, by another process, a condition unfavorable to organic mold.

These various conditions, which put organic mold in a state of suffering, gradually exert the following action on it:

When the mold constituting the organism is subjected to unfavorable causes that are too severe or of too prolonged duration, it tends to lose its organic type, that is to say, its organization, and return to its ancestral form; under these conditions, the organic mold does not die; it is its organization *and its simple organic properties that disappear*; *returning to its ancestral form, its development ceases to be subject to the influence of the nervous system and to obey the laws of the evolution of the organism's tissues.*

The changes in form that organic mold can undergo are numerous; they fall into two categories.

1. The transition of organic mold to the state of bacterial culture.

2. The transition of organic mold to other abnormal mycelial forms; these most often give rise, simultaneously, to abnormal bacterial elements in the body.

Transition of organic mold to the state of bacterial culture.

By stopping circulation in a limb for too long (tourniquet, experimental ligature), it causes gangrene. *Vibrio septica* and other bacteria appear in the subcutaneous connective tissue; some are autogenous, others heterogeneous.

Vibrio septica is a heterogeneous bacterial form, in an anaerobic environment, of a mold that causes a permanent mycosis: organic carrot mold.

Frostbite of a limb results in the same process and necrosis of the anatomical elements. Too high a temperature (burns) also does this. The action of prolonged cold and too high and prolonged heat manifest themselves in the same way on all living animal or plant organisms; it has been previously demonstrated that wet rot of the potato, due to an excess of water in the external environment, comes from the alteration of the organic mold of the tuber and its passage to the bacterial state; this wet rot reproduces easily, with the same bacterial forms, by subjecting the healthy tuber to frost or a temperature of 55°C for an hour or two, and then keeping it covered in crystallization.

The formation of spontaneous bacterial cultures occurs easily in cases where there is an effusion of blood or serosity (hematomas, traumas without a solution of continuity with the exterior, *surgical wounds)*, because the blood always, and normally, contains bacterial elements in vegetation intended for the formation of zymospores and leukocytes. The suppuration of a surgical wound is therefore not proof of a lack of asepsis or of an infection by heterogeneous germs; this suppuration has all the more chance to occur *when hemostasis is less well performed and the wound is less well cleared of extravasated blood.*

The experimental proof of the transformation of organic mold into a bacterial culture is very simple and can be done in multiple ways:

1. By grinding a fragment of any healthy tissue from the body and culturing it in broth.

2. By culturing a few drops of blood in broth.

3. By centrifuging oxalated blood and culturing a portion of the layer of leukocytes, hematoblasts, and spores deposited above the blood cells. Figures 5 and 6, plate 121, are two photographs of the bacterial elements of a horse blood culture obtained by this process.

4. By cultivating on agar the ground pulp of organs or fragments of connective tissue. We thus obtain both the gonidial forms of the animal's organic mold and some of its bacterial forms **(1)**. It is easy to verify that the molds collected are not accidental, because the same ones are always found in all individuals of the same species; the tissues of a mouse in Paris give rise to the same mold and the same bacterial forms as a mouse living in the fields 500 kilometers away.

If we cultivate the gonidial forms of organic mold in broth, we can obtain either a bacterial culture or enough bacterial elements to reproduce one by inoculation.

The finest ramifications of organic mold present, in all parts of the organism, spirillary or sinuous, or short and straight, appendages ending in spores. The disintegration of a tissue releases these elements as well as those of the segmented mycelial filaments; in any ulceration, for example, the mycelial endings of organic mold exposed and vegetating on the surface of the wound become disorganized in the form of the fusiform bacillus and the spirillus, elements always associated because they exist on the same mycelial filament, one carrying the other.

Organic mold vegetates in a special form in each organ; that of the nerve centers is clearly different from that of the liver, for example, although specifically linked to it; the result is that the bacterial forms obtained by culturing connective tissue are not the same as those obtained with liver tissue or brain tissue, but each form, being specifically linked to the others, can be reduced to them by appropriate culture procedures.

The bacillary forms obtained by culturing the pulp of tissues are extremely varied: Eberth's bacillus form, coli form, diphtheria form, vibrio forms, nail or pinhead forms, Koch's bacillus form, and so on.

None of these forms is characteristic of the species; the specific determination of a bacterium can only be made with certainty by transforming the bacterial culture into an aerial hyphomycete in one or more gonidial forms.

Since the coli form is a bacterial form of the organic mold of man, it should not be surprising that it is always found associated with Eberth's bacillus. It is also not surprising that, in many autogenous diseases, colibacillus is found in lesions and is also found in organ lesions caused by heterogeneous pathogens.

(1) *Bacterial cultures appear in the form of a creamy or opaline semi-liquid coating, forming numerous colonies.*

Bacillus botulinus is a segmented form of the organic mold that develops in the rotting meat of pigs.

The constant and normal presence of bacteria in the blood or organs has been known for a long time. They have been considered saprophytes and are believed to enter through the digestive tract. These bacteria are, in reality, outside of periods of active infection, elements of specific organic mold; when they are heterogeneous germs, they are not saprophytes, but pathogens. Indeed, these bacteria always result in the development of mycelial elements in the organs (long-term mycosis), and their presence cannot be tolerated without damage by the organism, *because they live at the expense of the tissues.*

Transition from the organized form of organic mold to other abnormal mycelial vegetative forms.

Organic mold, suffering as a result of defective conditions (nutrition, cold, insufficient circulatory activity, etc.), *can lose its organic type and take on another vegetative form, the development of which becomes autonomous and no longer takes place according to organic law.*

The characteristic of this new form is to multiply very actively and to form, unlike organic mold, a large mass of fruiting bodies of a new and abnormal type.

This change in vegetative form is the cause of autogenous diseases of the animal organism, tuberculosis, cancer, pneumonia, pulmonary congestion, and of various organs, rheumatism, neuritis, sciatica, etc., which we will briefly review.

Tuberculosis, cancer.

This change in vegetative form is the cause of two of the most serious human diseases: cancer and tuberculosis. It is demonstrated elsewhere:

1. That human tuberculosis is, at least in a large number of cases, spontaneous and determined by the development of an abnormal vegetative form of the organic mold of man; the vegetative transformation takes place in the period of organic misery (pre-tuberculosis) which precedes the period of active evolution; the new mold is necrotizing because *it lives at the expense of the normal, but not resistant, organic mold* which is in contact with it, and it develops an enormous mass of fructifications (spores and zymospores) which constitute the caseous mass of the tubers and the purulent matter which is evacuated outside.

2. That cancer is constituted by an abnormal vegetative form of organic mold characterized by the special form of its mycelium and its fructifications and especially by the independence of its development which no longer follows the laws of evolution of normal organic elements; this abnormal vegetative form extends and propagates, even to other points of the organism, by the zymospores which it develops and which reproduce it completely. The zymospores can easily, because of their Brownian motion, penetrate into the tissues neighboring the tumor, over a large area, and form new germinative masses there which will constitute new foci; it is by this process that they infect distant lymph nodes.

Tuberculosis and cancer are therefore two diseases of spontaneous, autogenous evolution; the abnormal molds that constitute them being specifically linked to human

organic mold, the human organism is, consequently, a medium of choice for their evolution; *this fact makes it evident that the reproductive elements of these abnormal forms can also develop in a new organism into which they penetrate by contagion.*

It is also evident that this development, if it can take place for tuberculosis, which is not in doubt, can also take place for cancer.

Pneumonia. Pulmonary congestion.

Pneumonia is due to abnormal vegetation of the organic mold of the lung. The viscous sputum of the pneumonic patient is made up of the hyaline matter of the culture expelled and coagulated in the bronchi, and containing the elements of the mold in vegetation; the pneumococcus is one of the segmentation elements of the mycelium contained in the sputum; the culture of these gives rise to the *Penicilium* form of the organic mold of man. Idiopathic pulmonary congestion is of the same nature.

Rheumatism.

Under the influence of various causes, among which cold appears to be the most important, the connective elements of the organic mold undergo a vegetative surge that constitutes chronic or even acute rheumatism.

Neuritis. Sciatica.

A vegetative surge of the mycelial elements that constitute the nerves is the cause of neuritis and sciatica; as in other organs, the mold constituting the nerve can change shape to develop a cancerous tumor (neuromas).

Arteriosclerosis.

The organic mold that constitutes the arterial walls can, like that of the tissues, change shape. An artery that has become sclerotic shows abundant and abnormally shaped mycelial vegetation in its wall, multiple large sclerotia, and a large quantity of spore-forming fruiting bodies. Examination of the wall shows that *the mold is in the process of returning to the ancestral type. In the nerve substance, the arterial wall sends out mycelial branches in all directions;* these develop into the neighboring tissue, multiply there, live at its expense and necrotize it; *this explains why the cerebral lesions of arteriosclerosis develop along the course of the vessels.* If we examine a softening focus of an arteriosclerotic, we see that the softened matter is constituted by an enormous mass of fructifications (spores) of the necrotizing mold mixed with mycelial elements in vegetation; if we examine the walls of the focus, that is, the area of extension of the softe-

ning, we see that it contains enormous sclerotia forming multiple mycelial trunks of large dimensions which extend their multiple branches in all directions. It is this mycelial vegetation which destroys the organic cerebral matter; the softened central zone of the lesion is not constituted by softened cerebral matter, but by elements of the new mold. A focus of softening is therefore essentially identical to an enormous tubercle.

It is easy to understand that the mold which thus constitutes foci of softening, by destroying the nerve substance, can easily destroy the vascular wall and cause hemorrhages in arteriosclerotics.

The development of abnormal mold in organs other than the brain can produce either analogous lesions or sclerotic tissue. The process I have just described is so clear that there is no doubt that it is indeed the direct cause of these lesions.

Blood diseases

Blood contains multiple elements whose vegetation can be deviated and become abnormal: red blood cells, hematoblasts, germinal masses (leukocytes), spores, zymospores, various bacillary elements.

Spores and bacterial elements can multiply abnormally and constitute spontaneous autogenous septicemia. Puerperal septicemia, which has been attributed without exception to an external infection, can arise, for example, from the development of normal bacterial elements of the blood remaining in the uterus after childbirth, the environment and temperature being most favorable to their multiplication; the zymospores of this abnormal culture can cross the uterine wall and infect the blood. It is therefore not certain that, in a case of puerperal septicemia, there was infection by the obstetrician and professional negligence.

Diseases resulting from contamination of the body by a heterogeneous mold.

It has been shown that bacteria have no individuality and are the segmenting elements or fruiting bodies (spores and zymospores) of molds.

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I have obtained the transformation, into their aerial hyphomycete form, of most of the known heterogeneous pathogenic elements; a photographic representation of this will be found later.

The heterogeneous infecting agent is always a mold; the parasites that have been described as hematozoa, protozoa, etc., do not have this nature. They are elements of a pathogenic mold.

It is demonstrated later that the malaria hematozoa, trypanosomes, spirillae, spirochetes, leishmanias, pyroplasmas, and amoebas are not protozoa, but constituent elements of pathogenic molds.

Diseases evolve in two phases: one acute, bacterial, the other chronic, mycelial.

It has already been explained above that the evolution of pathogenic molds, which penetrate the animal organism, occurs in two successive phases: the first, bacterial, of variable duration (generally four to twelve days), the second, mycelial.

In the bacterial phase, the elements of the pathogenic mold evolve like the elements of any in vitro culture in a liquid medium; the zymospores that form, develop intense chemical activity in the blood, *resulting in fever;* this decreases, then ceases, when the phenomenon of agglomeration, by becoming more pronounced, groups the zymospores in the germinative masses and thus suppresses their chemical activity.

Due to the richness of the blood in albuminoid matter, culture in *anima vili* forms numerous germinative masses much more easily than in vitro culture. These masses, *which constitute the heterogeneous leucocytes of the infecting agent*, vegetate in the first cycles of bacterial evolution by forming mycelial filaments which segment into bacteria; if bacilli are formed, these often continue their segmentation into micrococci. After a certain time, when the culture, very rich in zymospores (maximum of the febrile period), has formed a lot of hyaline substance (agglutinin) intended for the formation of the mycelium, the constitution of the germinative masses is accelerated and their size increases; they enter vegetation and emit, into the blood itself, long mycelial filaments. Because of their size and the congestion of this vegetation, they are arrested in the capillaries at a given moment and are only found rarely in the blood.

Arrested in the tissues, these masses develop their mycelium there; this is how the mycelial phase is established.

The rarefaction or disappearance of pathogenic bacteria from the blood does not mean, as we have seen, that they have been destroyed, but only that they have changed form.

This evolution can be verified very easily by examining the blood, then the organs, after inoculation with the culture of a heterogeneous pathogenic agent; the existence of the mycelial phase can be verified very simply, at any time, by culturing the pulp of the animal's organs; this culture always gives rise to the *Penicilium* form and other gonidial forms characteristic of the inoculated mold.

The late accidents of acute diseases are not caused by soluble toxins; but by the mycelial vegetation of the chronic phase.

Any heterogeneous mold infecting the animal organism establishes itself there in a lasting mycelial form, thus constituting a long-lasting, or even permanent, mycosis (syphilis). The most frequent diseases, smallpox, measles, scarlet fever, typhoid fever, diphtheria, cerebrospinal meningitis, etc., are followed, after their acute period, by various disorders (paralysis, liver, lung, and kidney disorders, trichophytosis, skin eruptions,...etc.), which have been attributed to the impregnation of the organs by soluble toxins. In reality, these disorders are caused by the mycelial vegetation of the pathogenic agent in the organs and are those of the chronic or mycelial phase of the disease.

The existence of toxins consisting of soluble and chemically definable bodies is not demonstrated by any conclusive fact. It has been shown that these toxins are composed of figurative, reproducible elements of the mold, the zymospores; the violent symptoms of the acute period of a disease are caused by the zymospores of the pathogenic agent, granulations endowed with an intense power of chemical transformation and which constitute the active agents of the ferments.

The existence of mycelial vegetation in the organs, for a long time after the acute period of illness, explains late accidents by the profound disorders that this vegetation can cause in the tissues and which can go as far as sclerosis of an organ or the formation of softening foci.

The disorders of the mycelial period of syphilis (gummy lesions) and those of malaria (lesions of the liver and spleen) are too well known for it to be necessary to dwell further on these facts.

Original source of viruses or heterogeneous pathogenic agents of contagious diseases of man and animals.

It was indicated at the beginning of this work how I came to know the original source of viruses.

Having transformed the most common viruses into gonidial forms of the molds that give rise to them, I recognized, by isolating the gonidial forms of the organic mold of food plants, that the pathogenic agent of typhus possessed forms identical to those of the organic mold of oats; shortly after, I was able to recognize that the organic mold of barley was identified with that of the pathogenic agent of diphtheria. I thus recognized, successively, that corn is the original source of the typhoid fever virus, wheat and rye those of the paratyphoid viruses A and B; rice that of malaria; the potato that of tetanus, vaccinia, smallpox, and foot-and-mouth disease; the tomato that of chickenpox; the lettuce that of measles; the carrot that of scarlet fever and rabies, the *Amanita phaloïdes* and citrina that of cholera, etc.

After having determined that animals and plants are made up of an organized mold, and after having isolated the gonidial forms of the organic mold of man, I was able to ascertain that these were identical to the gonidial forms that I had obtained by transformation of cultures of human tuberculosis. This therefore established that the original source of human tubercular elements is the human organism itself. The same conclusion resulted from the comparison of the gonidial forms of human organic mold with those provided by the culture of the pulp of cancerous tissues.

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By isolating the gonidial forms of animal organic mold and by comparing these forms with those of the pathogenic agents of certain diseases, I was able to recognize that the syphilitic mold is the organic mold of the monkey (macaque, cercopithecus, chimpanzee) and that the pathogenic agent of icterohemorrhagic spirochetosis is the *Aspergillus* form of rat organic mold.

Ongoing research will probably soon reveal the original source of other diseases, leprosy, glanders, anthrax, etc.

An important fact emerges from these determinations: when we examine the list of the most common food plants, we see that the most frequent human diseases are caused by the penetration into our bodies of bacterial elements from the organic mold of potatoes, tomatoes, carrots, lettuce, various species of cabbage, corn, barley, oats, rye, rice, oranges, lemons, and mandarins, etc.

But other food plants: chicory, salsify, lamb's lettuce, sorrel, watercress, beetroot, chard, cardoon, crosne, legumes (peas, beans, lentils, broad beans), eggplant, pumpkin,

celery, parsley, chervil, onion, garlic, shallot, leek, fruits, apples, pears, plums, strawberries, etc., all very easily give rise to a bacterial culture that must contaminate humans **(1).** I have isolated these bacterial cultures as well as the gonidian forms of all the plants just listed.

Physicians frequently encounter diseases of aberrant forms, which they are unable to diagnose. Among these diseases, there are some caused by the organic mold of the plants listed above. Potato diseases include smallpox, tetanus, and foot-and-mouth disease; scarlet fever is the disease of carrots, chickenpox that of tomatoes, measles that of lettuce, and diphtheria that of barley, exanthematic typhus that of oats..., etc.

It therefore seems certain that there is a disease of chicory, one of beetroot, one of peas, beans, one of leeks, etc. There are diseases of the potato and the tomato; it would be surprising if two other common food solanaceous plants, eggplant and chili pepper, did not also cause one.

There is therefore surely a whole series of human diseases that are still unknown, but less frequent than the diseases already cataloged and described. These diseases can only be recognized and cataloged, in turn, after isolation and identification of the pathogenic molds that are their agents. The study of these diseases is currently continuing.

The same observations apply to diseases whose original sources are animal organisms. It is quite certain that monkeys and rats are not the only animals whose organic mold can be pathogenic for humans. *We have seen that Bacillus botulinus is a bacterial form of the organic mold of pork.*

I have verified, by inoculation of guinea pigs, that the organic mold of fish (plaice, whiting, red mullet, eel, carp), amphibians (frogs), reptiles (caiman, turtle, gongyle), birds (peacock, sultana hen, parrot, parakeet), mammals (mouse, mole, sheep, horse, ox, dog, etc.), is very pathogenic. *The same is certainly true for humans.*

It is therefore certain that, as with plants, there are still unknown diseases caused by organic mold from animals that infect humans, either through the digestive tract or by inoculation from biting animals (lice, fleas, etc.).

(1) *These facts draw attention to a curious and frequent phenomenon: urticaria, which regularly occurs in certain people after any ingestion of a food material specific to each individual, strawberries or other fruits, certain fish or shellfish, and, for example: it is probably a rapid vegetative outbreak of an old mycosis, that of the organic mold of strawberries, for example, caused by the zymospores of raw or insufficiently cooked food.*

As a document and to help you judge the differences that exist between organic molds from animals and plants, I have attached to this work plates representing gonidian forms, and in particular the *Penicilium* form of the following species:

For animals: the horse (Pl. 309), the pig (Pl. 310), the sheep (Pl. 311), the mouse (Pl. 315), the peacock (Pl. 316), the sultana hen (Pl. 317), the parrot (Pl. 318), the tortoise (Pl. 319), the caiman (Pl. 320), the frog (Pl. 321), the eel (Pl. 322), the whiting (Pl. 328).

For plants, salsify (Pl. 324), *Eucalyptus globulus* (Pl. 325), *Amanita phalloides* (Pl. 326), *Amanita citrina* (Pl. 327), *Collybia velutipes* (Pl. 328), and *Lycoperdon pratense* (Pl. 329).

From this account, it follows that human diseases are: some, and the most serious, autogenous (due to an alteration of organic mold), and others due to heterogeneous parasitic molds whose original source is the organism of plants and animals or their decomposition products; the most common diseases among the latter are caused by the organic mold of the plants that are our daily food.

It is therefore almost impossible for us to escape these diseases, since our diet can only be made with plants or animals, or with substances extracted from them.

Our food is therefore made up, in its entirety, of substances that are a vegetative form of the pathogenic agents of our diseases and which can easily give rise to the bacterial culture of these agents.

If we consider that we absorb a large quantity of these materials every day, that they are destroyed by digestive enzymes, and that, despite this daily ingestion, cases of infection are extremely rare relative to the number of individuals, we must conclude that the fear of absorbing microbes is greatly exaggerated. It is obvious, due to the nature of our food, that if this ingestion were very dangerous, the existence of man and animal species would be very precarious. We ingest, without being able to avoid it, a large mass of microbes, but we digest them. It is very likely that, when a pathogenic germ develops in the body, it is because it has not been destroyed by digestion. Various conditions favor the penetration, through the digestive tract, of virulent bacterial elements that are not digested:

1. The ingestion of small quantities of uncooked substances, outside the secretion and presence of digestive ferments, that is, after the end of digestion.

2. The frequent penetration, into the digestive tract and with saliva, of bacterial elements produced by the fermentation of food substances that remain embedded between the teeth or in the gingival-labial space.

It is almost certain that it is this infection starting in the mouth that is the cause of the development of diphtheria and certain angines in many patients; it is certain that this is the sole cause of the development of thrush in infants and adults.

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Another cause of infection appears to lie in the ingestion of food, that has been cooked, for several days and fermented despite this cooking, or of fermented and undercooked food (seasoned meats), or of raw and spoiled foods. With these materials, the fully developed bacterial culture of the food is ingested.

This statement is not a hypothesis; rotten oranges, mandarins, or lemons, for example, contain the developed culture of the pathogen of Malta fever.

The bacterial culture of a food appears to contaminate humans more easily than raw food.

Knowledge of the original source of viruses of contagious diseases naturally leads to knowledge of the rules to follow to ensure their prophylaxis and reduce the frequency of cases of infection.

For this prophylaxis, it is necessary to distinguish between cases of spontaneous infection, *which are the starting point of epidemics,* and cases of secondary infection caused by transmission of the virus from patient to patient. It is obviously the disappearance of cases of spontaneous infection, that is, infection of the first patient or patients, that is important to seek out: knowledge of the original source of the virus makes it possible to establish rules that should prevent a large number of these cases of primary infection.

Let us cite some examples to clarify this point:

Smallpox is caused by a gonidial form of the organic mold of the potato; without Jenner's vaccination, smallpox would therefore be very common, with any housewife peeling these tubers without any precaution, having hands completely soiled by their juice or by the molds developed on their surface, and even putting her fingers to her mouth without any suspicion. It is certain that hand washing, precautions intended to not spill anywhere the water used to wash the tubers, which must be drained down the sink, and careful and sufficient cooking would have previously prevented a considerable number of cases of smallpox.

The same is true for the tomato, the agent of chickenpox, for the carrot, the agent of scarlet fever, and for lettuce, the agent of measles.

A large number of cases of diphtheria must be avoided by eliminating barley flour from children's food and by eliminating the use of barley straw as bedding for dairy cows, the milk being generally soiled by manure from the stable.

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It seems certain that the number of cases of rabies in dogs (or other animals) will be significantly reduced by avoiding giving them carrots as food, which often remain for several days in a bowl or on the floor of their kennels where they ferment.

Foot-and-mouth disease in cattle, whose pathogen is organic potato mold, should also decrease considerably in frequency by the application of simple rules. How could foot-and-mouth disease not be as frequent among cattle as smallpox was among humans, when, upon entering a farmyard, one sees rotten tubers on the manure and even scattered on the ground alongside peelings, and the housewife throws on the ground, *at the very place where the cows pass, the murky water in which she has just washed the potatoes for the next meal?* Since these mistakes are daily occurrences, the cows' hooves and the humans' shoes inevitably carry into the stable the elements of a culture of the foot-and-mouth disease agent.

One of the most important points in the fight against major epidemics is to know the original source of the virus that causes them. We will see, in the study of cholera, that knowing the original source of the virus makes it possible not only to stop an epidemic at its beginning, *but to predict its imminence before it appears and to take preventive precautions that will prevent the contamination of individuals.* Indeed, the pathogenic agent of cholera is the bacterial culture of poisonous amanitas, notably Amanita citrina and Amanita phalloides. These mushrooms always rot normally on the ground, and rain accelerates this rot. A short, violent rain is enough to carry the bacterial culture developed by the rotting of these mushrooms into rivers or drinking water pipes; this is the immediate cause of the development of cholera epidemics.

We therefore know in advance that, in a hot and humid year, when there is a proliferation of poisonous amanitas, a cholera epidemic could follow a heavy rain or continuous rain; it will therefore be possible to take the necessary precautions, the use of boiled water for all domestic purposes, when we only observe the proliferation of amanitas and, even more so, when heavy rain occurs during this proliferation during the months of July, August, September, and even in certain years during the month of October.

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We will now approach the study of human diseases, with a view to determining the forms of the pathogenic agents that cause them, and their original source. We will study, first, the autogenous diseases of the organism, cancer, tuberculosis, pneumonia, etc., then the various diseases caused by heterogeneous molds.

We will not discuss here a certain category of the latter, serum diseases, for which it was previously determined that the pathogenic agent is the organic mold of the animal from which the serum emanates; it is, moreover, almost exclusively, in this case, serum disease and mycosis caused by horse serum, that is, the organic mold of this animal.

TUBERCULOSIS

At the beginning of this study on tuberculosis, I recall that it has been previously established that *in any animal or plant organism, the elements of a living tissue can, under certain conditions, change shape and transform into a bacterial culture*. This has been demonstrated for the potato parenchyma; the cells of this parenchyma transform into a bacterial culture when they have been previously subjected to the action of frost or a temperature of 50 to 55.

The mechanism of this transformation is simple; let us first recall that bacteria are the segmenting elements of the mold mycelium; the anatomical elements of animals and plants *are organized forms of the ancestral specific mold*; they lose their organization when subjected to adverse influences (cold, excessive heat, insufficient nutrition, etc.); by losing the normal organized form, the specific organic mold returns to its ancestral form and takes on either the bacterial or the mycelial type; its vegetation becomes independent of that of the tissues and escapes the laws of their development.

The research that led to the knowledge of these facts thus established that, without any external contamination, a bacterial culture or an abnormal mycelial culture can develop spontaneously in the animal organism, and that the element that gives rise to them is the organic mold subjected to a cause of decay.

These facts will allow us to arrive at an understanding of the nature and origin of tuberculosis.

Nature and Origin of Tuberculosis

Knowledge of the nature and origin of tuberculosis is given by the following facts:

1. Cultures of human or bovine tuberculosis can be transformed into an aerial mold affecting various gonidial types.

2. The caseous matter of tubers can be transformed into an aerial mold affecting various gonidial types.

3. A characteristic tuberculosis is induced by inoculating the guinea pig with the spores of the gonidial forms of the molds obtained by transformation of tuberculosis cultures or caseous material from tubers.

4. The gonidial forms resulting from the transformation of human or bovine tuberculosis cultures and caseous material from tubers are identical to those obtained by transformation of the specific organic mold of humans or cattle.

5. Certain vegetative forms of human organic mold, cultivated in vitro and derived from healthy, normal tissues, confer tuberculosis on guinea pigs.

The elements constituting the normal tissues of the organism, the caseous material from tubers, and the cancerous tissue, can, by appropriate culture, be transformed into identical elements or into the same bacillary form.

6. The tuberculosis virus is made up not only of Koch's bacillus, but of all the elements of the caseous or tubercular matter constituting the tuber: leukocytes, giant cells, mycelium, spores, and bacilli. It can be made up of these elements without containing bacilli.

7. The constituent elements of tuberculosis cultures, or Koch's bacillus cultures on potato or agar, or in a veil on glycerin broth, are the same as those of the caseous matter of the tubers.

8. The tuberculosis element being the specific organic mold diverted into its vegetative form, man carries within himself the element necessary for the spontaneous development of tuberculosis; the causes which provoke this development are those which determine the state of physiological misery of the organic mold.

9. The tuberculizing element being the specific organic mold diverted into its vegetative form, the human organism is an eminently favorable environment for its growth, either spontaneously or through contamination.

10. The tuberculizing element being specifically linked to the organic mold of man, any healthy human organism must be sensitive to the elements from human tuberculosis cultures that constitute tuberculin.

These facts, the demonstration of which will be provided, establish:

1. That human tuberculosis is often spontaneous.

2. That, in all cases, with the exception of cases of tuberculosis of bovine origin and, obviously, of contagious origin, it is caused by the vegetation of elements of human organic mold deviated in its form and properties.

3. That Koch's bacillus is neither the principal causal agent nor the characteristic element of tuberculosis. The most characteristic element of the latter is the germinative mass, its mycelium, and its fructifications.

Let us now proceed to the demonstration of the facts that justify these conclusions.

1. Transformation of tuberculosis cultures into aerial molds (hyphomycetes)

I obtained this transformation:

1. With six different human tuberculosis cultures, two of which were made available to me by Professor Legroux of the Pasteur Institute, and four by Doctor G. Kuss.

2. With two bovine tuberculosis cultures and one avian tuberculosis culture made available to me by Professor Legroux.

The transformation of cultures into hyphomycetes can be achieved by two processes:

1. By inoculating the original culture onto various solid media: egg yolk, cooked beef, casein, or high-sugar agar (5% maltose, 5% glucose).

The cultures are kept at laboratory temperature and in the light. The process is lengthy ; transformation is generally achieved on one of the inoculated media, but after a very variable time (from eight days to two months or more).

Transformation generally occurs in the gonidial types *Sterigmatocystis* or *Penicilium*. As soon as a mold colony has developed, its spores are inoculated onto high-sugar agar, where they develop very quickly.

2. By inoculating a guinea pig with the original culture and growing the pulp of the animal's organs on agar: liver, spleen, lung, lymph nodes. A massive dose can be inoculated, which quickly kills the animal. The culture of the lung tissue becomes covered, from the third or fourth day, with colonies of the *Penicilium* form of the inoculated culture. At the same time, other gonidial forms of this culture are generally obtained: *Sterigmatocystis, Aspergillus, Monosterigmata*, etc.

This process is rapid and reliable; the *Penicilium* and *Mucor* forms of the guinea pig's organic mold are distinct enough to easily avoid confusing them with the previous ones.

At first, I obtained the *Penicilium* form only in a rudimentary state, as shown in plates 162 *(Penicilium* form of human tuberculosis) and 168 (bovine tuberculosis). But, on the other hand, I obtained other gonidial forms which allowed me to clearly differentiate human tuberculosis from bovine tuberculosis.

I obtained, with the cultures of human tuberculosis, a *Mucor* form (Fig. 1, 2, 3, plate163), and an *Aspergillus minimus* form (Bainier) (Fig. 4, 5, plate 163).

Bovine tuberculosis cultures yielded, in addition to the *Penicillium* form, a *Mucor* form (Figs. 3, 4, 5, 6, pl. 169) clearly distinct from the *Mucor* form of human tuberculosis, a *Monosterigmata* form (Fig. 2, pl. 169), and an *Aspergillus* form (Fig. 1, pl. 169).

The study of the *Aspergillus minimus* form (Bainier) showed that it is only a rudimentary type of the gonidial form *Sterigmatocystis.* I obtained this same form by culturing on agar the caseous material of human lung tubers.

This study therefore established, at that time, a clear specific distinction between human tuberculosis and bovine tuberculosis and provided information on the nature of aspergillus tuberculosis; these are constituted by the vegetation of a gonidial form of *Aspergillus* having the same origin as other tuberculosis: the specific organic mold. Subsequently, I succeeded in obtaining the *Penicilium* form in its complete development by successive subcultures of the primitive cultures and passage through the guinea pig.

I will not establish, here, a distinction between the *Penicilium* forms of human and bovine tuberculosis cultures; this distinction is very difficult, and I limit myself to showing the various varieties of fruiting bodies that can be observed in the two species. The *Penicilium* forms of human tuberculosis are contained:

1. In plates 162, 164, 165, for a tuberculosis culture from Professor Legroux, of the Pasteur Institute.

2. In plate 166, for a culture designated Ay, from Dr. G. Kuss.

3. In plate 167, for a culture designated Lacr, from Dr. G. Kuss.

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Various types of the *Penicilium* form of bovine tuberculosis are contained in Plates 168, 170, and 171 and were obtained with a culture from Professor Legroux of the Pasteur Institute, mentioned as having been isolated by Professor Vallée of the Al-fort Veterinary School.

Plate 172 contains the *Penicilium* (rudimentary types, Figs. 1, 2), *Sterigmatocystis* (Figs. 3, 4), and *Monosterigmata* (Fig. 5) forms of avian tuberculosis. The *Sterigmatocystis* form is clearly distinct from that of human tuberculosis; the *Monosterigmata* form is very distinct from that of both bovine and human tuberculosis.

From these facts, it follows:

1. That the tuberculosis cultures currently maintained in laboratories (cultures on glycerin-coated potatoes, cultures on agar or egg yolk, veil cultures on glycerin broth) are cultures of a mold that can easily be transformed into other vegetative or gonidial forms (*Penicilium, Aspergillus, Sterigmatocysti, Monosterigmata*), either directly by changing the culture media or indirectly by passage through the guinea pig's body.

2. That aspergillus tuberculosis is of the same nature and origin as other tuberculosis and differs only in the vegetative form of the tuberculating agent.

3. That human, bovine, and avian tuberculosis is due to molds of different species that can be distinguished from each other by comparing their various gonidial forms.

2. Transformation of the caseous matter of the tubers into hyphomycetes.

In a fragment of tuberculous lung, the caseous matter is taken from a large caseous tubercle and spread on sugar agar; the culture being kept at laboratory temperature, it is first observed that the caseous matter vegetates and multiplies. If a fairly large number of cultures have been made (12 to 24), the appearance, on some of them, and after a variable time (eight days to two months), of molds of variable appearance, white, gray, ochre, brown, light yellowish-green, or dark green, is observed. White and grey molds, which emit spores along their filaments, generally do not have a well-characterized gonidial type. The other molds are of the following types:

1. *Monosterigmata Type* (Fig. 13, 14, pl. 173). Brown-colored mold, whose fruiting bodies have the form of short appendages, generally swollen at their base or in the middle, ending in a ball and emitting strings of large spores; the appendages arise along the filaments, perpendicular to their direction.

2. *Cephalosporium acremonium Type* (Fig. 15, 16, pl. 173). Moss-green mold; it is of great interest, because the inoculation of the spores of the culture consistently determines a characteristic tuberculosis.

3. *Aspergillus minimus type* (Fig. 9, 10, pl. 173). Green or yellowish-green mold. This form is identical to that already obtained by direct transformation of human tuberculosis cultures; it is a rudimentary form of the Sterigmatocystis type.

4. *Sterigmatocystis type* (Fig. 11, 12, pl. 173). Light yellowish-green mold.

5. *Acaulium type* (Fig. 7, 8, pl. 173). Light ochre or light buff mold. It is of great interest because it evidently constituted the tubercular mold in a very distinct case of tuberculosis that I studied; in this case, the lung tissue presented a particular yellowish or ochre tint when cut; the caseous matter presented the same light ochre tint; Twenty-four culture tubes of lung fragments were made; in all of them, the *Acaulium* type grew

to the exclusion of any other mold; it was therefore this type which vegetated in the lung, because, usually, it is the other types of mold which develop, the *Acaulium* form being rare: the one which is represented in figs. 7 and 8, plate 173, relates to another case of tuberculosis and was obtained by culture of caustic material which, on the other hand, had given rise to the types *Monosterigmate, Aspergillus minimus, Sterigmatocystis* and *Penicilium.*

6. *Citromyces type* (Fig. 2, 3, 4, 5, 6, plate 173). Green mold; it is a rudimentary form of the *Penicilium* type. This *Citromyces* type, grown on Endo agar, turns it pink, like the *Citromyces* form obtained by direct transformation of B. coli cultures.

7. *Penicilium* type (Fig. 1, pl. 173). Green-colored mold identical to that obtained by direct or indirect transformation of usual tuberculosis cultures on glycerin media.

Other gonidial types also develop on the cultured material, notably the *Sporothricum, Mucor,* etc. types.

The aim of this study was to show that the caseous material of the tubers contains elements capable of giving rise to all the gonidial forms obtained by transforming pure tuberculosis cultures into hyphomycetes. In certain cases, it appears that the caseous material is constituted by a mold of a specific gonidial type (case cited of vegetation of the *Acaulium* form).

I also obtained the ordinary *Aspergillus* gonidial form (*Aspergillus glaucus*, de Bary form) by culturing fragments of lung affected by normal tuberculosis. This confirmation confirms the nature of *Aspergillus* tuberculosis and the origin of the mold that grows in the lesions.

3. Characteristic tuberculosis or diffuse pulmonary lesions are induced by inoculating the guinea pig with gonidial forms derived from the transformation of tuberculosis cultures or from the caseous material of the tubercles.

These gonidial forms are all very virulent for the guinea pig and kill it in small doses, generally in six to twenty days. They cause either typical tuberculosis or congestive pulmonary lesions, often intense, without apparent formation of tubercles. The result varies with the gonidial form inoculated.

With the gonidial forms *Monosterigmata, Cephalosporium acremonium*, and *Aspergillus minimus*, inoculation of a small quantity of spores by the intraperitoneal or subcutaneous route regularly causes intense congestive pulmonary lesions, sometimes with fairly large caseated areas, and also constantly the formation of large tubercles 2 to 3

millimeters in diameter in the liver and spleen; the form that appears to be the most virulent is the *Aspergillus minimus* form.

I have also obtained characteristic tuberculosis by inoculation of spores of the *Penicilium* form, either from ordinary tuberculosis cultures (by transformation) or from the culture of the pulp of tissues from tuberculized guinea pigs. The *Penicilium* culture that gave me the most characteristic results was obtained in this way:

Following the next step of a tuberculous human lung, *a hard, non-caseated tubercle* is isolated from an area that has not yet caseated; it is crushed in a watch glass and inoculated into a guinea pig, which dies after twenty-three days and at autopsy presents generalized tuberculosis with a multitude of fine tubercles of the lung, liver, and spleen, and a few caseated tubercles the size of a small millet grain. Culture of the pulp of these organs on agar gave rise to the *Penicilium* form, which was used for subsequent inoculations.

Here are, as examples, the results of an experiment carried out with each of these types of molds:

I. *Inoculation of the Penicilium form*

Injection into a guinea pig under the skin of the leg of about half a cubic centimeter of salt water holding in suspension a small quantity of *Penicilium* spores, obtained by culturing the lung tissue of a guinea pig made tubercular by inoculation with the pulp of a non-caseated tubercle from the human lung. Died after seventeen days.

Autopsy: Congestive pulmonary lesions of the lung. Tuberculosis of the liver and spleen (multitude of small tubercles of 0.5 to 1 millimeter) and of the peritoneum. The peritoneum lining the diaphragm and abdominal walls shows numerous tubercles. Mesenteric lymph nodes large and caseous.

II. *Inoculation of the Cephalosporium acremonium form*

This gonidial form was obtained on a small fragment of tuberculous human lung cultured on agar. Subcutaneous inoculation of a small quantity of spores suspended in salt water. Death after five days.

Autopsy: Intense congestive pulmonary lesions. Intense hepatic congestion with numerous and large caseous tubercles (about twenty visible on the surface of the organ). Large spleen, distended by large caseous tubercles, about ten of which are visible on the surface.
The same lesions are seen in other animals that died at a longer time (three weeks).

III. *Inoculation of the Aspergillus minimus form*

Form obtained by direct transformation of the caseous substance of a tubercle of the human lung, cultured on agar.

Intraperitoneal injection into a guinea pig of a small quantity of spores suspended in salt water. Death after twenty days.

Autopsy: Massive tuberculosis of the lungs, three-quarters of which are caseated. Liver and spleen presenting a considerable quantity of caseated tubercles 2 to 3 millimeters in diameter (large spleen).

IV. *Inoculation of the monosterigmatous form*

Form obtained by direct transformation of the caseous material of a tubercle of the human lung cultured on agar. Inoculation, into the subcutaneous cellular tissue of the paw, of a small quantity of spores suspended in salt water. Death after 22 days.

*Autopsy: I*ntense congestive lesions of the lung. Intense liver congestion with large caseous tubercles. Large spleen with numerous caseous tubercles measuring 1 to 3 millimeters. Very numerous small caseous tubercles on the visceral and parietal peritoneum. Large and caseous mesenteric lymph nodes.

Thus, pure cultures of tuberculosis on potato or glycerin broth and the caseous material of the tubercles can be transformed into hyphomycetes and various gonidial types; these, when inoculated into the guinea pig, can give it tuberculosis, like the original cultures or like the caseous material in nature.

On the other hand, the caseous matter of the tubercles developed in the organs of the guinea pig by these inoculations *reproduces tuberculosis when inoculated;* I have reproduced these tuberculosis in five or six successive reinoculations of caseous matter from the tubercles.

Often, inoculation of the guinea pig with cultures of *Penicilium* resulting from the direct transformation of the caseous matter, or with pure cultures of tuberculosis, causes

only intense congestive lesions of the lung without the formation of tubercles in the liver or spleen or only with rare tubercles.

This fact is not surprising, because it is repeated in the same way when the caseous matter of human pulmonary tubercles is inoculated in nature. It often causes only congestive lesions. On the other hand, by inoculating the substance of hard tubercles, not

yet caseated, crushed and suspended in salt water, a generalized tuberculosis is caused with the formation of very numerous tubercles.

It seemed to me that, for *Penicilium* cultures, the property of forming tubers diminishes, either by increasing the age of the culture or by increasing the number of generations. *Penicilium* cultures, derived from products with a high tuberculizing power, a hard tuber for example, have a greater tuberculizing power than those derived from the caseous material of tubers, whose tuberculizing power is less. For other gonidial forms, for example the *Cephalosporium* form, I observed that the tuberculizing power was preserved after three years.

Let us note here the fact that it is the gonidial forms *Monosterigmata* and *Cephalosporium acremonium* that cause the most intense tuberculization.

Tuberculosis can therefore be caused by the growth in the body of hyphomycete spores obtained by transformation of in vitro tuberculosis cultures or of the caseous material of tubers; it develops, *although the inoculated material does not contain Koch's bacilli.* The relationships that exist between these hyphomycetes and Koch's bacillus will be studied later. Currently, we will research *the origin of the tuberculizing agents,* guided in this study by knowledge of the forms of their various gonidial types; this research reveals that *the gonidial forms of tuberculosis cultures or of the caseous material of tubers are those of the organic mold specific to humans.*

4. The gonidial forms resulting from the transformation of cultures of human or bovine tuberculosis and the caseous matter of tubers are identical to those obtained by transformation of the organic mold specific to humans.

To obtain the gonidial forms of the organic mold specific to humans, I cultured the pulp of the organs (liver, spleen, lung, brain) of full-term fetuses that died, either at the time of expulsion or within twenty-four hours following. I repeated these cultures on five subjects.

In all five cases, and regardless of the organs, it was always the same mold or its identical gonidial forms that were obtained.

Plates 174 and 175 represent different aspects of the gonidial form *Penicilium.* These forms are found identically in the previous plates (Pl. 162, 164, 165, 166, 167) containing the various types of the *Penicilium* form of human tuberculosis.

Plate 176 contains the *Citromyces* (Figs. 1, 2, 3, 4, 5, 6, 7), *Aspergillus* (Figs. 8, 10), and *Sporothricum* (Figs. 9, 11, 12) forms of the human organic mold.

The *Citromyces* form represented here is identical to the one I obtained by transformation of a culture of *Bacterium coli* from humans.

As for the *Sporothricum* form, it bears enormous fruiting bodies, and this gonidial type could very accurately be compared to the *Peronospora* type of organic mold in plants. I have obtained this same *Sporothricum* type several times, by culturing the pulp of cancerous human tissues on agar.

The same identity is observed between the gonidial forms of organic mold in beef and those of bovine tuberculosis cultures.

Plates 177 and 178 contain the various forms of the gonidial types *Penicilium* (Figs. 1 to 16, pl. 177; figs. 1 to 12, pl. 178), Mucor (Figs. 13 and 14, pl. 178), and *Rhizopus* (Figs. 16, 17, pl. 178) of the organic mold of beef. The same forms of the *Penicilium* type obtained by transformation of bovine tuberculosis cultures are found in plates 170 and 171. In addition, I had obtained, by transformation of bovine tuberculosis cultures, a *Mucor* form (Figs. 3 to 6, pl. 169), of a type very distinct from that given by human tuberculosis cultures (Fig. 1, 2, 3, pl. 163).

The fact that the *Mucor* form obtained by transformation of the organic mold of beef is identical to that obtained by transformation of bovine tuberculosis cultures, confirms the nature and origin of the latter at the same time as it explains the differences observed by bacteriologists between cultures of human tuberculosis and those of bovine tuberculosis.

The *Rhizopus* form, obtained by transformation of the organic mold of beef, is a mold of a very dark brown color, almost black. I obtained a *Rhizopus* form of the same color and similar shape by transformation of the organic mold of sheep. I have never observed it among the transformation forms of the organic mold of man.

These facts lead us to understand why we observe in humans a specific tuberculosis, in cattle a specific tuberculosis, and in chickens a specific tuberculosis. It has been thought that these are cultures of tuberculosis adapted to the organism of an animal species by a long series of successive transmissions to individuals.

In reality, tuberculosis is, as a general rule, always the same in an individual of the same species, because it is a form of transformation of the organic mold characterizing that species.

5. Vegetative forms of the organic mold of humans cultivated in vitro and derived from healthy, normal tissues confer tuberculosis on the guinea pig.

The elements constituting the normal tissues of the organism, the caseous matter of the tubers, and the cancerous tissue can, by appropriate culture, be transformed into identical elements or into the same bacillary form.

I naturally had to try to transform the organic mold of man into cultures identical to those of human tuberculosis and to reproduce tuberculosis in animals by inoculation of these cultures.

The study of this question, which is in progress, has not yet given me all the indications it should provide. But it has nevertheless already yielded results of capital importance. I obtained the transformation of human organic mold into an in vitro culture of globular elements identical or similar to polynuclear and mononuclear leukocytes (the latter being an evolution of the former).

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This culture forms on broth (usual glycosylated peptone) a very rapid development veil (in twenty-four or thirty-six hours) and gives, in the animals to which it is inoculated, a typical tuberculosis with caseous tubercles of the liver and spleen and massive congestive lesions of the lung; it was obtained in the following way:

The lung tissue of a fetus that died at term is reduced to a pulp and spread on sugar agar; the cultures are stored at 23-24°. After four to five days, the cultured tissue gives rise to a creamy substance that spreads over the agar. This substance is transferred to broth, or better, to an Erlenmeyer flask in which sugar agar has been solidified in a region of the conical wall (2 to 3 centimeters wide).

Broth is introduced to a height of one centimeter and the creamy substance is inoculated onto the agar one centimeter above the surface of the broth; when the creamy substance reaches the surface of the broth, it develops into a veil.

I obtained a similar veil with the lung of two different fetuses. The elements constituting this veil are represented in Plate 179 (Gr. 1100, figs. 1, 2, 3, for the veil obtained with the lung of one fetus; figs. 4, 5, 6, for the other); they are identical to mono- and polynuclear leukocytes and to the globocellular elements of cancerous tissue. The veil formed from elements of this nature develops very quickly. Inoculated in a matrass, it covers the entire surface of the broth in thirty-six to forty-eight hours.

I have tried to understand the process of formation of these elements. Plate 180 represents their form after ten hours (Figs.1,2,3) and thirteen hours after seeding(Figs. 4, 5, 6). Figures 1, 2 and 3 show that, quite clearly, these elements are formed according to

the general mode of formation of germinative masses in bacterial cultures. The first germinative masses seeded emit mycelial filaments which segment into bacterial elements. These, in turn, reconstitute by their agglomeration new germinative masses which will form a new cycle of evolution. It appears, in Figures 3, 4, 5, that, in addition to this mode of formation, the germinative masses are capable of forming others by budding.

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The bacterial element which, by its fusion in the germinative masses, comes to constitute the nuclear part, is a bacillus which can be strongly stained by basic colors (Fig. 3, pl. 180). *This bacillary element is of great interest, because it can be obtained by two other processes: 1st by broth culture of the caseous matter of the tubercles of the human lung; 2nd by broth culture of the pulp of the cancerous tissue.*

Figure 7 of Plate 179 represents this bacillary element obtained by culturing, in broth, the pulp of a breast cancer. The preparation photographed in this figure was made with a three-day culture, stored at room temperature. In the culture, large germinative masses develop, emitting large mycelial trunks from which finer ramifications emerge, which, through their segmentation, give rise to the bacillary elements. Small round or oval germinative masses are also formed, similar to those formed in cultures originating from the fetus. It is the study of the formation and evolution of the elements in this culture of cancerous tissue that was the origin of the research contained in this work.

The culture of the caseous matter of the tubers also makes it possible to obtain both this same bacillary element and globocellular elements identical to those that form the veils of cultures originating from fetal tissue.

Figure 8, Plate 180, represents these globocellular elements formed in a broth culture of caseous material from the tubercles of the human lung. Caseous material removed from an unopened tubercle located in a non-cavernous region was inoculated onto the agar placed on the side wall of a flask containing broth and one centimeter above the level of the broth. The culture being placed in an incubator at 30°, after three days, a veil developed on the surface of the broth, the primitive constituent elements of which are represented in Figure 8, Plate 180; these elements are germinative masses analogous to those which form in the veils of cultures of fetal tissue (Figs. 3, 4, 5, Plate 180).

In addition, the culture already contained larger germ masses emitting large mycelial branches. Later, this veil underwent a more advanced evolution than the fetal tissue cultures. It gave rise to large, highly branched mycelial trunks.

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The elements of this veil are represented in plate 181. The large mycelial branches are, some very rich in basophilic protoplasm and highly colorable (in black in the figures),

the others very little colorable (pale grey in the figures); this difference comes from the fact that all the branches emit fine branches (laterally and especially towards the extremities) in which all the basophilic protoplasm of the large branches flows, until they are almost totally deprived of it; these fine basophilic branches are segmented into basophilic bacterial elements which are noted in all the figures of plate 181.

A general law applies here: every mold mycelial filament, whether organized or not, is initially basophilic; the basophilic protoplasm of the germinative mass flows through the mycelial filaments toward the ends and will constitute the basophilic protoplasm of the segmentation elements (bacterial elements) of the terminal mycelial filaments.

These bacterial elements here take two main forms: 1st, the form of the basophilic bacillus already observed in cultures of fetal tissues and cancerous tissues (Fig. 4,pl. 181); 2nd, the form of coccobacilli (visible in all the figures).

There can be no question of assigning fixed dimensions to these bacilli and coccobacilli. Every terminal mycelial branch segments into bacilli or coccobacilli regardless of its size. We can therefore find, in the same form, elements three or four times larger than others. The large mycelial trunks themselves, once deprived of their basophilic protoplasm, segment in turn and release enormous elements that can also be described as bacterial (Figs. 1 to 6), some of them 20 μm long and 4 to 7 μm wide. In the preparations, the growth of the fine branches on the large trunks is clearly visible and we observe them in the process of segmenting, either into bacilli or coccobacilli.

* * *

These facts naturally led me to think that tuberculosis can be caused in an animal by inoculation or ingestion of the organic mold of another animal.

A series of experiments in which guinea pigs received by intracerebral inoculation an emulsion of normal dog spinal cord, ground in sterile water, gave the following results:

one animal, out of eight inoculated, suffered from generalized tuberculosis with tuberculous peritonitis; five others presented either tubercles of the lung or tubercles of the liver and spleen.

Repeating the same experiment on eight guinea pigs with an emulsion of normal rabbit marrow, one animal suffered from generalized tuberculosis with tuberculous peritonitis and two others presented tubercles of the liver.

In a third series of experiments, guinea pigs received by intraperitoneal or subcutaneous inoculation an emulsion of gray matter from the cerebral hemispheres of the ox. In two out of six cases, two or three large tubercles (2 to 3 millimeters in diameter) formed on the liver and spleen.

In these three series of experiments, only the animals in which the formation of tubercles was observed are mentioned; but, for the others, the brain matter proved equally virulent and consistently caused rapid death with congestive lesions of the lung and lesions of the liver and spleen (congestion or cyrrhosis, hypertrophy).

In a fourth series of experiments, the brain matter of the ox was given to guinea pigs by stomach ingestion, mixed with food. A piece of ox brain, the gray and white substance of the hemispheres, is ground in salt water to form a homogeneous emulsion. The bran given to the animals is moistened with this emulsion. All the guinea pigs thus fed died after twelve days to a month, some with massive and intense congestive lesions of the lung only, two others with characteristic lesions One of them, who died sixteen days after ingestion, presented, in addition to massive congestive lesions of the lung, hypertrophied mesenteric lymph nodes, the size of a walnut (packet 25 millimeters in diameter) and caseous. The other, who died eighteen days after ingestion, presented, in addition to caseous mesenteric lymph nodes (the size of a small hazelnut), massive congestive lesions of the lung with a caseous island the size of a pea, and a characteristic tuberculosis of the liver and spleen, entirely invaded by a considerable number of caseous tubercles 1, 2, or 3 millimeters in diameter.

The caseous matter of these tubercles, successively inoculated into three other guinea pigs, caused them to develop identical tuberculosis.

These facts therefore show that tuberculosis can be transmitted not only by inoculation of tuberculosis cultures, which are a form of transformation of animal organic mold, but also by inoculation or direct ingestion of normal organic mold constituting tissues or organs, the brain for example.

In summary, the organic mold of humans or animals can lose its organic type and evolve into different vegetative forms. It can evolve into globo-cellular or leukocyte elements, germinative masses which, depending on their development, give rise to either cancerous tissue or tuberculous elements. It will be demonstrated later that with fetal spleen tissue, cultures of typical cancerous elements can be obtained that reproduce without changing form by successive inoculations.

It has also just been demonstrated that the culture of either cancerous tissue pulp or caseous material from tubers allows identical cellular or bacillary formations to be obtained.

The demonstration of the nature and form of the primitive tubercular elements, and the nature of tuberculosis itself, is completed by the fact that typical tuberculosis is

determined in guinea pigs by subcutaneous or intraperitoneal inoculation of the leukocyte elements of the veil of human fetal lung tissue pulp cultures.

Here are two experiments as examples.

Experiment 1. A guinea pig receives a small quantity of broth veil elements from a human fetal lung tissue pulp culture by subcutaneous inoculation (hind paw). It dies sixteen days after inoculation. At autopsy, it shows a considerable quantity of tubercles, about one millimetre in diameter, in the liver and spleen, and massive congestive pulmonary lesions.

Experiment 2. A guinea pig receives an intraperitoneal inoculation of a small quantity of the elements of the veil on broth from a pulp culture of the spleen of a fetus. It is shown elsewhere (Pl. 200) that these elements present the form of elements typical of cancerous tissue. The animal dies fifteen days after inoculation. At autopsy, it presents numerous caseous tubercles of 1 to 2 millimeters in the liver and spleen, and massive congestive lesions of the lung.

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Thus, the inoculated elements presenting either the type of leukocytes or the type of cancerous elements (sarcomas) and originating, by culture, from fetal tissues, lung or spleen, determine a typical tuberculosis in the guinea pig. The caseous matter of the tubercles of the inoculated animals reproduces the same typical tuberculosis in four or five successive series of animals to which it is inoculated.

This fact sheds light on the development of tuberculosis and the tubercle. It is established, by histological research (Kiener, Borrel, etc.), that the tuberculous cell always derives from a lymphatic cell and that giant cells are formed by the fusion of large mononuclear leukocytes.

In rabbits, after intravenous ingestion of an emulsion of tuberculous bacilli, it is observed, according to Borrel, that, from the second day, clusters of bacilli and poly- morphonuclear leukocytes *have formed at certain points, to which large mononuclear leukocytes with numerous expansions are added; on the third day, some of these large leukocytes fuse to form giant cells.*

These large mononuclear leukocytes, exhibiting numerous expansions, are germinative masses beginning to emit their mycelium; they are not leukocytes belonging to the inoculated animal, the rabbit, but germinative masses formed by the tuberculating element itself, that is, heterogeneous leukocytes; they are therefore the tuberculating element itself, that is, a new form taken, in its evolution, by the inoculated bacillus. The giant cell is a large germinative mass formed by the fusion of smaller germinative masses. These are the normal phenomena in the evolution of any bacterial culture in vitro:

the bacterial elements fuse there to constitute germinative masses which are true leukocytes.

The tuber is therefore a formation resulting from the evolution of a bacterial mass; this mass forms the usual leukocytes of any bacterial culture and giant cells resulting from the fusion of several leukocytes: these leukocytes and giant cells are the germinative masses which will emit the mycelial vegetation found in the tuber, vegetation bearing the usual fruiting bodies of molds (spores of various sizes) and giving rise to bacterial elements by segmentation.

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6. The tuberculosis virus is made up not only of Koch's bacillus, but of all the elements of the caseous or tuberculous matter constituting the tuber; it can be made up of these elements without containing any tubercle bacilli.

There is therefore no need to distinguish, in a tuber, Koch's bacillus, which would be the tuberculizing element, from the other elements that constitute the caseous matter, that is, leukocytes, giant cells, mycelial filaments, and granulations (spores). In reality, these various parts are all constituent elements, but with distinct forms and roles, of the tuberculosis virus.

This conclusion is not hypothetical. It is easy to verify the existence of these various elements in the caseous matter of a tuber. There we find giant cells or other smaller germinative masses, the mycelium they emit, fruiting bodies (spores of various sizes) and bacterial elements. Plates 182 and 183 represent the photographs of these various elements. We can succeed in observing the entire mycelial framework of the tuber by the following procedure: with a very fine spatula, we remove from a single mass all the caseous matter of a tuber in the process of caseating but not completely softened; we place it in a watch glass containing distilled water and we shake it carefully; the spores and granulations spread in the liquid and, after some time, only the mycelial framework remains which is spread on a slide either by dissociation with needles or by crushing; we dry, we fix by heat and we stain.

Figures 1 and 2 of Plate 182 represent a part of the mycelial framework of a tuber thus prepared and stained with dahlia; germinative masses are also noted there (Fig. 1). By studying Figure 1 with a magnifying glass, one will easily observe more or less large rounded fructifications carried at the end of lateral appendages or short branches emanating from the mycelium. The gonidial form which vegetates in the tubers, at least in the most common forms of tuberculosis, is therefore a *Monosterigmate* form.

Plate 183 contains photographs of the usual elements of the caseous matter of the tubers (Figs. 1 and 2) and of the elements of the hard tuber not yet caseified (Figs. 3,4,5).

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To observe the first ones, it is enough, after having carefully stirred a little caseous matter with water, to spread, dry hot and color with dahlia; for the latter, the material contained in a hard tuber is extracted and crushed between two glass slides; it is dried and colored with dahlia. Figure 1 shows germinative masses of various sizes, including a large one with a huge single round nucleus and emitting several very distinct mycelial filaments (Gr: 1100 D). Figure 2 (Gr: 1100 D) also shows germinative masses of various sizes of caseous material and, very distinctly, the numerous mycelial filaments they emit.

Figures 3, 4, 5 (Gr: 610 D) show the germinative masses of various sizes constituting a hard tuber, not yet caseated. We notice the same enormous central nucleus.

The mycelial branches emanating from the germinative masses emit laterally fine branches bearing fine spores (zymospores) of 0.3 to 0.6 µm. But it is mainly very small germinative masses of 1 to 3 µm or even large spores that emit fine branched filaments covered with zymospores. These masses and their mycelial branches become entangled with each other to form veritable thick bushes. Figure 9 represents one of these masses at a magnification of 1100 diameters; the zymospores are very clearly seen, borne laterally and almost sessile, on the sinuous filaments that emerge from the mass.

These masses and their free zymospores constitute a large part of the volume of the caseous matter.

These zymospores constitute the fermentative element of the tuberculating agent, or more precisely, of the tuberculous matter; they are the active (or most active) element of tuberculin; their existence is easily observed in all tuberculin; they are animated by a lively Brownian movement.

In the hard tubercles of humans and in the spleen of the tuberculous guinea pig, I was able to observe that Koch's bacilli are formed by the segmentation, either of fine mycelial filaments emitted by the germinal masses, or of the finest ramifications of the large branches that emanate from them.

Thus, in summary, the elements that constitute the tubercle are:

1. The germinative mass which can affect any size, from 1 to 40 or 50 u and more, and whose role is to emit mycelial filaments which will give rise to zymospores, then to bacterial elements (Koch's bacilli or other forms) when they segment.

2. The mycelial filaments emitted by the germinative masses.

3. The fruiting bodies, large spores of 1 to 3 µm formed on the mycelial filaments of the large germinative masses, or fine spores of 0.3 to 0.6 µm carried, either on the finest

ramifications of the large mycelial branches, or on the fine mycelial branches emitted by the smallest germinative masses (from 1 to 24) or even by the large spores when they germinate directly.

4. Bacterial elements: these elements are either Koch's bacillus, or basophilic bacillary elements, or coccobacilli, the latter analogous to the various bacterial elements represented in Plate 180.

These various elements are therefore constituent parts of the same organism and have the same relationships between them as the seeds, fruits, leaves, branches, trunk, and roots of the same tree.

Koch's bacillus is therefore not the characteristic element of tuberculosis. It is only one of the parts of the tubercular mold, and its presence is inconstant. This mold can vegetate by giving rise to its other usual elements, without forming Koch's bacilli, or by forming bacilli of other forms than coccobacilli, or basophilic bacilli.

7. The constituent elements of tuberculosis cultures or cultures of Koch's bacilli on potato or on agar, or in a veil on glycerine broth, are the same as those of the caseous material of tubers.

The proof of these facts can be given without the slightest doubt. Indeed:

A. *The elements of the tubercular mold, other than Koch's bacillus, such as germinal masses, spores of all sizes, giant cells, mycelium, small germinal masses of 1 to 3 µm forming the zymospores, which exist in the caseous matter of tubers, also exist in the usual laboratory tuberculosis cultures* (cultures on potato, agar, or in veil on broth).

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Figures 6, 7, 8, plate 183, represent photographs of the elements of a culture of human tuberculosis on potato (culture designated Ay, origin: Doctor Kuss) preserved at 38°. These elements are difficult to observe because they are very fragile and, moreover, agglomerated in clusters, stuck together, in the culture. I was only able to observe them isolated by crushing between two glass slides a piece of culture the size of a millet grain. Many elements are simply dissociated from the others and escape crushing. In Figure 6, we see a group of three large oval germinative masses, one of which has retained one of the mycelial branches it emits; Figure 7 contains a group of two or three germinative masses emitting mycelial filaments. In Figure 8, we find a germinative mass fragmented by crushing and of which only a part remains with its large spherical basophilic nucleus. In addition, we distinguish in Figure 7 fine sinuous branches bearing zymospores and emanating from the germinative masses. In the preparations, there is a multitude of spores or small masses of 0.8 to 2 or 3 µ emitting one or more fine filaments bearing zymospores.

B. *Any usual culture of tuberculosis on potato, agar, or broth, preserved and accustomed to a temperature of 38°C, can be transformed into a culture of another vegetative type by growing it on another medium at room temperature or at 22°-24°C.*

I performed this transformation on Dr. Kuss's culture, designated Ay. I obtained a first transformation, at the very beginning of the research reported in this work, by transferring this culture to carrots and preserving it at a temperature of approximately 18°C; I obtained abundant mycelial development with segmentation into coccobacilli.

At that time, I believed in contamination, because no known facts allowed me to believe in such a transformation; this was explained to me later by other facts and I was able to easily obtain, from that moment on, another transformation characteristic of the same culture; To do this, simply inoculate a potato culture plot in a matrass on the surface of peptone broth, glycosylated and glycerinated or not, and keep the culture at room temperature or at 22-24°. A very thin veil develops very slowly on the surface of the broth. Figures 3 and 4, plate 182, are photographs of this veil, in the process of developing, fifteen days after inoculation.

In figure 3, we notice germinative masses emitting mycelial filaments; some of these bear lateral appendages (filament on the right and at the top of the figure) which are found identically in cultures of the developing *Monosterigmata* type; others bear clearly formed sterigmata ending in a round spore; several are partly visible in the central part of the figure. This also contains basophilic bacilli and elongated or short coccobacilli, some of which bear one or two polar globules. Some of these coccobacilli are barely colored.

In Figure 4, in addition to the preceding elements, we notice numerous small granular masses which are leukocytes or germinal masses at the beginning of their formation.

Thus, a typical culture of Koch's bacillus can easily be made to form elements identical to those found in the caseous matter of tubers and, on the other hand, elements (coccobacilli) analogous to those that characterize zoogleic tuberculosis.

Following these facts, let us recall that Ferran, then Auclair, succeeded in transforming cultures of *Koch's bacillus* into cultures of a bacillus exhibiting the form and coloring reactions of *Bacterium coli*. Ferran says he obtained, conversely, by serial inoculations, the return of this bacillary form to the *Koch's bacillus* form. There can be no doubt that these facts are correct, because:

1. The cultivated human organic mold gives rise to a *Bacterium coli*.

2. The transformed *Bacterium coli* of human origin (culture of Dr. Legroux, Pasteur Institute) gives rise to a mold of the *Citromyces* type.

3. The caseous matter of human lung tubers, transformed by culture into hyphomycetes, gives rise to a *Citromyces* form identical to that which comes from *Bacterium Coli* of human origin, and which, like it, turns Endo agar pink.

4. Human organic mold transformed by culture gives rise to a *Citromyces* form identical to that which comes from the transformation of the caseous matter, that is, from the transformation of human *Bacterium Coli.*

The observation of these facts leads us to formulate again. These are already old conclusions, little known, but accurate; they are those already stated by Vignal and Malassez **(1)** and the substance of which is as follows:

1. Tuberculous lesions without bacilli can produce bacillary tuberculosis by inoculation, which indicates that the phymatogenic parasite already exists in them, but not in the bacillary form.

2. These same lesions can produce non-bacillary but zoogleic tuberculosis.

3. By inoculation of *Zooglea*, the bacilli can appear after several generations; it therefore seems that the bacilli and *Zooglea* are "different forms of the same parasite."

These facts correspond very exactly to those demonstrated just now: the tubercular element exists not only in the bacillary and zoogleic form, but in other multiple forms.

It exists in variable bacillary forms that must correspond to the microbes of Eklund, Aufrecht, and Baumgarten; it also exists in the form of round spores corresponding to the micrococcus isolated by Toussaint and with which he obtained tuberculous lesions by inoculation.

There can therefore now be no doubt that establishing the diagnosis of tuberculosis by the exclusive observation of the presence of Koch's bacillus, in tuberculated tissues or in sputum, constitutes an error. The other elements of the tubercular mold always exist in the tubercular matter alongside Koch's bacillus; while the latter may be missing, the other elements, germinal masses, mycelium, spores, and zymospores, are never missing.

(1) *VIGNAL ET MALASSEZ, Soc. de Biol., November 5, 1883.*

8. Origin of Tuberculosis.

The tubercular element being the organic mold deviated in its form:

A. *Man carries within himself the element necessary for the spontaneous development of tuberculosis.*

B. *The human organism is an eminently favorable environment for the growth of tubercular mold when it enters through contamination.*

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There can be no doubt that the development of tuberculosis is spontaneous in humans for a high proportion of tuberculosis patients.

It has been demonstrated that the animal or plant organism is constituted by a mold and that this, under unfavorable conditions, can deviate in form and undergo various transformations, either into bacterial cultures, or into elements of other forms, or into cancerous tissue.

Other facts have taught us:

1. That the tuber is constituted by the elements of a mold.

2. That this mold is specifically the same as human organic mold and is derived from it.

3. That an in vitro culture of human Koch's bacillus is a vegetative form of human organic mold.

These new facts being known and compared with clinical observations, we arrive at the conclusion, as a certain number of clinicians have already done, that the state of pretuberculosis is indeed the period of physiological misery of the organism during which the tissues, not yet tuberculous, undergo a reduction in their capacity to resist the development of tuberculosis. We can add, now, with the help of new notions, that *it is the period during which the organic mold, in a state of physiological misery, undergoes the transformation, which makes it lose its organic type and changes it into a tubercular mold.* In this case, tuberculosis is therefore spontaneous and there is no need for contamination from outside to cause it; similarly, an infection of another nature can develop spontaneously, without contamination, at any point in the organism following a trauma, the action of cold, etc.: The bacterial element is created from scratch in the lesion by the organic mold.

Experimentation and observation have clearly demonstrated that human tuberculosis is contracted by contagion and that it can pass from a sick subject to a healthy subject who lives in contact with it.

From this obvious contagion, Villemin drew an accurate deduction in concluding that tuberculosis is caused by an inoculable agent; he drew an incorrect conclusion in concluding that it must be included in the class of virulent diseases, alongside syphilis and glanders; his conclusions necessarily implied the false notion that tuberculosis is always of contagious origin and cannot develop without contagion.

It now appears that the reasoning opposed to Villemin by Pidoux was correct when he wrote **(1)**:

"Experiments on animals give you this or that result, and, instead of controlling them by clinical experience and by all the data of human physiology, you construct on them a general doctrine of human tuberculosis and of all diseases. To do this, you overturn all acquired notions. We must accept, from one day to the next, that phthisis falls from the clouds and that, in its pathogenesis, the subject, the constitution, the hygienic conditions, heredity, the diatheses, are nothing, and that everything is on the blade of a lancet loaded with an impossible tuberculosis virus, undoubtedly coming from a tuberculosis sufferer who inherited it from another, and so on up to the first man, who nevertheless inherited it from no one and must have formed it from scratch."

Later, the discovery of the tubercle bacillus by Robert Koch consolidated Villemin's error. From then on, and to this day, tuberculosis has been considered to be necessarily due to contamination by Koch's bacillus.

These erroneous notions have had a preponderant influence on the results of the fight against tuberculosis; they have directed the fight primarily towards avoiding contagion; they have masked the indication that could direct the fight against the spontaneous development of tuberculosis, that is, the fight against the physiological misery of the human organism, the causes of which are overwork, insufficient nutrition, alcoholism, and diseases of contamination by heterogeneous germs.

The state of physiological misery can be created by a chronic illness that leads to weight loss and causes digestive disorders that hinder eating; such illnesses are chronic because the infecting germ continues to vegetate in the body permanently, in the form of a mold (permanent mycoses). The vegetation of the infecting mold in an organ creates conditions unfavorable to the organic mold. These facts explain why tuberculosis can appear as a distant consequence of certain illnesses, even acute illnesses that include an unnoticed chronic phase.

The current concept of the fight against tuberculosis is well condensed in these few sentences from Calmette's book: *Bacillary Infection and Tuberculosis*:

(1) *Bull. Acad. de Méd., 1867-1868.*

''Eminent masters have ingeniously condensed into lapidary formulas, intended for the public more than for doctors, some notions that they considered particularly useful to disseminate, for example: Alcoholism is the breeding ground for tuberculosis (Landouzy). Tuberculosis is caught on zinc (Hayem). Tuberculosis is an illness of poverty and ignorance (Landouzy), etc.''

''I think it is better not to repeat these aphorisms too often to the crowds, because they tend to divert attention from the essential goal that we must and want to pursue, which is to kill or render harmless the sources of infection.''

It appears, in the statistics published to date, that anti-tuberculosis prophylaxis has lowered mortality from tuberculosis to a certain proportion which now no longer appears to be decreasing appreciably. It is to be expected that *this proportion must include, for the most part, spontaneous tuberculosis,* that is, cases whose development is not subject to anti-tuberculosis prophylaxis, but only to *the fight against the tuberculizable terrain.*

It appears that only the fight against the tuberculizable terrain, that is, against spontaneous tuberculosis, will be able to significantly reduce the proportion of tuberculosis patients. The success of this fight depends above all on the elimination of the two principal causes of physiological misery: overwork and insufficient nutrition.

The proportion of individuals who are believed to have minimal tuberculous lesions, or simply infected by Koch's bacillus living in them in a latent state, is certainly not as high as one might believe; it has been considerably exaggerated by a reaction to which a meaning that it does not have and which is hypothetical has been attributed: the Pirket skin reaction or tuberculin reaction considered as a means of reliably detecting, in an apparently healthy individual, a latent and unsuspected tuberculous infection.

9. Value and significance of the Von Pirket reaction.

Numerous statistics published so far inform us about the proportion of positive reactions to tuberculin in presumed healthy subjects.

It appears that, in comparing the results of these statistics, various elements, notably the manner of carrying out the skin reaction, the dose of substance, the quality of the tuberculin, must seriously influence the results obtained; it is certain that, among these, the best are those which have the highest proportion of positive reactions. Various authors have obtained a proportion of 90 percent of positive reactions in subjects not known to have tuberculosis.

The statistic of Letulle and Grivez **(1)**, which appears to be one of the best, indicates, for example, 89 percent of positive reactions in 740 subjects aged over 15, subjected to the test and chosen at random from all social backgrounds in the city of Lille among those who did not attend hospitals or dispensaries. It is probable that by insisting on the search for the reaction in subjects who presented a negative reaction, the proportion of 89% would have been increased.

Despite this, the number of nine individuals reacting out of ten apparently healthy subjects indicates that, above the age of 15, almost all subjects react to tuberculosis. Does this fact mean that the subjects who react are surely carriers of tuberculous bacilli or lesions?

This conclusion is unfounded and is not established by the facts, particularly by a material test. The fact that, out of one hundred confirmed tuberculosis patients, all react positively does not allow us to conclude that tuberculous lesions exist in apparently healthy subjects who react positively. Such a conclusion is purely hypothetical, if it is not verified by the physical existence of the lesions.

In fact, the Pirket reaction is positive in subjects who, at autopsy, reveal no trace of bacillary lesions; the conclusion that they nevertheless carry bacilli is purely hypothetical.

Constitution of tuberculin.

To fully appreciate the significance of Pirket's reaction, it is necessary to know the nature of the elements that give tuberculin its activity. These elements are zymospores from the tuberculosis culture. They exist in countless quantities in the culture broth; the preparation of tuberculin eliminates from this broth the larger elements of the

culture, but does not deprive it of the zymospores which pass through the filters. The presence of zymospores in the tuberculina is easy to observe under the microscope; it is enough to add a little dahlia solution which colors the zymospores and allows them to be easily seen, between slide and coverslip, agitated by their lively Brownian movement; they have from 0 µ 3 to 0 µ 6 on average.

Tuberculin is therefore simply the toxin of the tuberculosis culture; its constitution does not differ from that of the diphtheria or tetanus toxins, and it is the same elements, the zymospores, which give it its toxic power; it differs from these latter toxins only by the treatment it has undergone and the temperature of 100° to which it has been subjected.

(1) *LETULLE, Paris Thesis, 1912.*

For many species, the temperature of 100° does not kill the zymospores and does not remove their power to reconstitute the initial integral culture. This question has already been addressed in the general study of toxins. I reconstituted the tetanus mold using only its zymospores heated to 120°. We know that this temperature kills tetanus spores with difficulty; it is not the spores, but the zymospores that retain their vitality.

I saw a filtered culture from the first breast cancer I studied completely reconstitute itself, using only zymospores, after heating for thirty minutes at 130°C; the reconstitution is very slow and only takes two or three months; the elements of this culture were elements derived from the human organic mold, like the elements of tuberculin of human origin.

It seems certain that the zymospores of tuberculin have retained their vitality. It is known that tetanus and diphtheria toxins injected into guinea pigs quickly reproduce the normal elements of diphtheria and tetanus cultures and that, with the help of these, it is possible to reconstitute even diphtheria and tetanus molds.

Tuberculin or tuberculous toxin must therefore, like the preceding ones, vegetate rapidly when inoculated under the skin of animals or healthy humans and form the usual elements of cultures in the animal organism, notably germinative masses (leukocytes); microscopic examination has, in fact, revealed the presence, in the region subjected to the effect of tuberculin, of numerous polymorphonuclear leukocytes, then mononuclear. These leukocytes were considered to be those of the organism flowing into the region under the influence of tuberculin.

The example provided by the results of injections of diphtheria and tetanus toxins indicates that the same must be true here and that the leukocytes appearing in the tuberculinized region were formed from scratch by the zymospores of tuberculin. Control of this fact is under study.

The formed elements of tuberculin must therefore enter into vegetation in both a healthy subject and a tuberculous subject and produce the characteristic effect. The reaction itself is therefore not characteristic and does not allow us to conclude that a reacting subject is surely the carrier of a bacillary lesion.

The only fact that appears characteristic, for confirmed tuberculosis patients, is their much greater sensitivity to the action of tuberculin.

In summary, the Pirket reaction loses its specific significance due to the fact that the active elements of tuberculin are the zymospores of tuberculosis cultures, elements endowed with both fermentative and vegetative powers and capable of developing and

multiplying, that is, of making any healthy organism react, as would any other inoculated virulent germ.

What could have led to the likelihood of the results of the Von Pirket reaction was the belief in a single origin of tuberculosis by contamination and ignorance of the spontaneous birth of tubercular elements in the human organism and their specific link with organic mold, combined with a certain exaggeration of the frequency of contamination by tubercular germs.

If nine out of ten apparently healthy individuals carried latent tubercular lesions, if the risks of infection were so great that virtually no one escaped them, how could the human species resist such a scourge?

It will only be possible to judge accurately and without exaggeration the importance of the risks of contamination when we have been able to approximately assess the proportion of spontaneous tuberculosis.

It is striking to note, by examining the statistics, that in the countryside and even in the cities, the prophylaxis of infectious diseases with heterogeneous germs has *considerably* reduced the proportion of cases of these diseases, while anti-tuberculosis prophylaxis has produced only insignificant results.

Knowledge of the contagiousness of tuberculosis and the principles of anti-tuberculosis prophylaxis are, however, fairly well known in general, especially in cities, for an

appreciable result to have been obtained. If prophylaxis has not produced the expected results, it is mainly because it was expected to produce results that it cannot: the reduction of the proportion of spontaneous tuberculosis, on which it is ineffective. Against the latter, the elimination of conditions unfavorable to organic mold and the care necessary for its normal development can only be effective. Elimination of overwork, alcoholism, suitable and sufficient nutrition, these are the treatments judiciously and rightly recommended by clinicians of the last century with a view to modifying the *tuberculizable terrain.*

Value of the means of diagnosing tuberculosis.

Apart from clinical procedures and radioscopic examination, the means used to recognize tuberculosis are:

1. The tuberculin reaction carried out with its different modalities (cuti-reaction, intradermal reaction, ophthalmo-reaction).

2. The search for Koch's bacillus in products known to be tuberculous.

3. Inoculation of guinea pigs with products presumed to be tuberculous.

The significance of the Von Pirket reaction has just been studied; a positive reaction in no way signifies that an individual is carrying a tuberculous lesion, however minimal; it only signifies that the elements capable of evolution contained in the tuberculin have evolved in the healthy subject tested.

The search for Koch's bacillus in tuberculous products is only meaningful when it is positive. Tuberculant elements always exist in tuberculous products, even in the absence of Koch's bacillus, in the form of germinative masses, mycelium, spores (micrococci), and zymospores. It is therefore the presence of the latter, *constant in tuberculous products,* that should be sought. But this search seems impossible because any organic product originating from an organ or tissue contains elements of the same shape, and nothing in the appearance of the tuberculant elements indicates their nature.

The positive result of inoculation in the guinea pig obviously creates a presumption of tuberculosis, but not a certainty; *inoculation with elements of normal organic mold is likely, as demonstrated above, to develop tuberculosis in the guinea pig.*

The transformation of normal elements of organic mold into tuberculant elements, which occur spontaneously in humans under certain conditions, can also occur in the guinea pig when inoculated.

The method of controlling by inoculation of animals is therefore likely to give an erroneous result.

Indications provided by new knowledge relating to the treatment of tuberculosis or immunization against this disease.

These indications concern:

1. Preventive treatment of tuberculosis.

2. Treatment by tuberculinization.

3. Treatment by pneumothorax.

4. Chemotherapeutic treatment.

5. Serotherapeutic treatment.

6. Immunization trials against tuberculosis.

1. Preventive treatment of tuberculosis.

The most important new knowledge that has to be brought into play in the treatment of tuberculosis is the spontaneous creation in the organism of the elements of the tubercular virus by transformation of the elements of the organic mold in a state of suffering. This knowledge confirms that the orientation of preventive treatment towards the modification of the tubercular terrain is indeed the path that can prevent the development of tuberculosis; it tells us that the tubercular terrain, that is to say the organism in a state of suffering or decay, is in no way already tuberculized or infected by the tubercular elements as is claimed; it is only on the path that will lead to the transformation called tuberculization; the designation, by the word pretuberculosis, of the state of decay that precedes the development of tuberculosis, is therefore very accurate.

Preventive treatment for these spontaneous tuberculosis cases must therefore ensure suitable, healthy, and abundant food, rest and the elimination of overwork, the elimination of alcoholism, and the improvement of the hygienic conditions of individuals and homes.

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It must, in addition, aim to combat the adverse effects of chronic, often unsuspected, conditions that put the body in a state of permanent suffering, for example the distant effects of heterogeneous pathogenic germs which, after apparent complete recovery from the acute illness, continue to inhabit the body in mycelial form (Example: typhoid fever, diphtheria, eruptive fevers, etc.). This is the preventive treatment recommended by current clinicians as likely to prevent the development of tuberculosis.

2. Treatment by tuberculinization.

Another concept that should be brought into play in the treatment of tuberculosis is that which establishes the nature of the active agent of tuberculin; this is the toxin of in vitro tuberculosis cultures; its active principle is the zymospore, the agent of tuberculous fever and the chemical agent (ferment) ensuring the development of the tubercular mold.

I have no new facts that would allow me to formulate a justified assessment of the value of treating tuberculosis with tuberculinization. However, since the worsening of the lesions accompanied by fever is due to a vegetative push of the tuberculinizing mold with proliferation of zymospores, it seems very doubtful that the introduction into the organism of new zymospores in tiny quantities, compared to those already existing, is likely to cause a lesion to reverse. It is known, moreover, that when introduced into the organisin in certain proportions, still minimal compared to those con-

tained in the tuberculized organism, tuberculin causes the development and immediate aggravation of the lesions, *that is to say, accelerates the growth of the tuberculinizing mold.*

Knowing the active element of tuberculin and its relationship with the tubercular mold, how can we admit that a small quantity of active elements can exert a favorable effect while a larger quantity has a harmful effect?

3. Treatment of tuberculous lesions of the lung by pneumothorax.

During the very numerous inoculations of pathogenic germs that I have carried out in guinea pigs with a view to transforming bacterial cultures into hyphomycetes, I have

observed in a rigorously constant manner that the preferred environment for the growth of bacterial cultures and molds is the lung, that is, the organ in which bacteria or molds find the most favorable conditions for oxygenation.

This fact indicates that oxygenation is favorable to the development of tubercular mold and that its reduction is likely to hinder this development.

The pneumothorax, which, by the retraction of the lung, has the double effect of reducing both oxygenation and the coefficient of blood irrigation, probably owes its favorable action to these two factors; the retrogradation of the lesions must be due to the unfavorable effect exerted on the growth of the tubercular mold by the reduction in oxygenation.

It is also to be presumed that muscular activity and movements that have the effect of accelerating pulmonary circulation and increasing ventilation, that is, oxygenation, must be unfavorable to the tubercular patient because they activate the vegetation of the tubercular mold. It seems that the most favorable condition for the tubercular patient must be as little pulmonary ventilation as possible.

It is probable that the rest cure, with the body lying down, acts by achieving these conditions and not only by reducing organic expenditure to a minimum.

4. Chemotherapeutic Treatment.

The fact that the organism is made up of an organized mold, and that the tubercular mold is a form of transformation of the former, *makes it impossible to apply the action of a chemical product to the latter without it manifesting itself on the former.*

Assuming the resistance of the two molds to be equal, we see that it is impossible to kill the tubercular mold or disrupt its growth without exerting the same action on the

normal organic mold. In the latter, we must distinguish between the resistance of the organization and the resistance of the ancestral mold to destruction; any adverse action, chemical or physical, exerted on the organic mold affects its organization before killing the ancestral mold. Deep frostbite of a foot destroys the organization of the tissues, but does not kill the organic mold, which continues to vegetate and constitutes the necrotic or rotten mass.

The destructive action of a chemical product can therefore only be exerted in the organism by first affecting the organic properties of the normal mold, a fact which will tend to favor its transformation into tubercular elements at the level of the lesions. Such a treatment therefore goes against the desired goal; one must conclude that all chemical products capable of destroying the tubercular mold or of hindering its vegetation are impossible to use because, used at the necessary dose, they would destroy the organization of the anatomical elements before reaching the tubercular mold. They can therefore only exert a harmful action.

These are indeed the results produced by the use of certain chemical products, creosote, for example.

But it may be quite different with chemical products that, on the contrary, have the effect of promoting mold growth; it is possible that such products may, through their favorable action on normal organic mold, contribute to stopping its transformation into tuberculous elements, that is, to stopping the progression of tuberculosis.

5. Serotherapeutic treatment of tuberculosis.

The question of antituberculous serotherapy has been discussed at length previously; there is therefore no need to develop it again here. I will simply point out that antituberculous serotherapy proves impossible:

1. Because a curative serum only acts on the progression of the bacterial or acute phase of a disease, which it shortens, but not on that of the mycelial phase, and tuberculosis is a chronic disease with mycelial progression.

2. Because the human tubercular element is a vegetative form of the human organic mold, and it appears difficult to create antibodies protecting humans against their own organic mold and its various vegetative forms. This explains the total failure of all attempts at serotherapy made to date.

6. Immunization trials against tuberculosis.

This question has also already been studied previously. I will therefore simply recall the main points.

Immunization against a disease, smallpox for example, involves giving the body a long-lasting fungal infection (to confer on the organism a long-lasting mycosis); the presence of the disease in its chronic mycelial phase ensures protection against the acute period of a new invasion by achieving agglutination and the rapid passage of new infecting elements to the mycelial state. There can therefore be no question of protecting against the mycelial phase of a disease, since it is the very existence of this phase that constitutes immunization. It is therefore not possible to immunize against tuberculosis, which is a dangerous disease due to its mycelial phase and since this, instead of immunizing, destroys the organism.

Apart from these facts, the specific link that exists between the tuberculous elements and the human organic mold appears to make immunization against vegetative forms derived from it impossible.

It was believed that Koch's phenomenon was seen as proof of the possibility of immunizing against tuberculosis. It has been explained elsewhere that this phenomenon does not have this significance.

It therefore appears that immunization against tuberculosis is impossible, whatever route it is attempted.

* * *

CANCER

Cancerous tissue is constituted by an abnormal vegetative form of the animal's specific organic mold; this, deviated in its organic form and properties, no longer obeys the laws of organic mold development; its growth becomes independent.

Cancer is therefore constituted by a deviation from the normal vegetative form of the specific organic mold; its initial cause is the one that provokes this deviation.

The lineage of the research that led me to knowledge of the nature of cancer was explained at the beginning of this work. It was indicated that while studying a cancerous breast tumor, I isolated, in the microbial form, the agent constituting the cancer; the study of the evolution of this microbial culture led me to knowledge of the origin and nature of bacterial elements, their evolution into hyphomycetes, and then the nature and formation of white blood cells.

After observing the formation of white blood cells in vitro in microbial cultures, another series of observations showed me that, in hyphomycetes cultivated in liquid media, certain mycelial elements can take on forms identical to those of the anatomical elements of cancerous tissues.

With this knowledge, I undertook the study of a series of varied cancerous tumors that were made available to me by Doctor Laval and Professor Desmaret.

The study of this series of tumors allowed me to establish that cancer *is always* caused by the growth of a mold in the animal organism. Evidence of this nature of cancer was provided:

1. By the isolation and culture of the cancer-causing agent from eighteen cancerous tumors.

2. By the identification of the anatomical elements of these tumors with the various elements of the molds.

Having obtained these results, I concluded that cancer is always caused by the growth of a *heterogeneous mold* in the body.

This conclusion was reached because, at that time, I was unaware of the nature of the tissues of the animal body. I therefore sought the origin of the heterogeneous molds that grow in the animal body.

Knowledge of the transformation of bacterial cultures into hyphomycetes, the observation of the existence of mold vegetation in the organism in the mycelial form in infectious diseases, led me to believe that the passage of these diseases to the chronic state coincides with the passage of the infectious agent to the stable mycelial state and with the establishment of a chronic mycosis.

Searching the organs for the presence of molds determining these mycoses, I noted that, always, in both healthy and sick animals, mold elements identical to those I had detected in cancers are found in all organs. This fact led me to conclude that all healthy individuals are carriers of *permanent and constant mycoses* from which they generally do not suffer, or suffer very little. We will see, in the study of tuberization in certain plants, that botanists have drawn identical conclusions for plants.

The fact that living beings could be thus constantly infected in all their organs by a considerable mass of parasitic molds without apparently suffering from them seemed improbable to me and prompted me to study the question at greater length. This study of molds in organs has been described previously. As we have seen, it showed me that there is no element in the animal organism (nor in plant organisms) that is not an element of mold, and that living organisms are entirely constituted by an organized mold.

This knowledge, once acquired, modified the significance of the facts I had previously established about cancer. I was thus led to resume the study of this question in a new direction. The various gonidial forms of the organic mold of man having been isolated, I compared them with the gonidial forms that I had obtained by culturing cancerous tumors; I was thus led to observe that these latter forms were identical, in almost all cases, to those of the specific organic mold of man.

This fact decisively confirmed that cancer is constituted by the vegetation of a mold and completed this conclusion by specifying that this is the specific mold constituting the organism. The examination and identification of the anatomical elements of cancers, made later, demonstrated that it is an abnormal vegetative form of the organic mold that develops to constitute cancerous tumors.

I was later to obtain another, equally decisive proof of the nature of cancer: I succeeded in transforming the normal elements of human fetal tissue into stable cultures of the elements characteristic of cancer (sarcoma).

In summary, the demonstration of the nature of cancerous tissue was established:

1. By transforming the mold constituting the cancerous tissue into one or more of its gonidial forms of aerial hyphomycete and by comparing them with the gonidial forms obtained by culturing normal human tissue, that is, with the gonidial forms of the normal specific organic mold.

2. By studying the nature and forms of the elements that constitute cancerous tissue.

3. By identifying these anatomical elements with similar formations developed by molds vegetating in vitro in liquid media or on very humid solid media.

4. By studying the forms taken by the elements of the normal specific organic mold in the liver, spleen, and other organs, and their comparison with the forms of the anatomical elements of cancerous tissue.

5. By the experimental transformation of normal elements of fetal tissue into elements typical of cancerous tissue.

The forms taken by the elements of organic mold in organs have been described at length in previous chapters.

I therefore assume them to be known, as well as the special forms, also described, taken by hyphomycetes (mycelium and fruiting bodies) in liquid or semi-liquid culture.

We therefore only have to deal in this chapter with the forms of the anatomical elements constituting cancerous tissue, their comparison with the forms of the elements of organic mold constituting normal organs or tissues, and their transformation into hyphomycetes.

CULTURE OF CANCEROUS TISSUES. TRANSFORMATION OF THE MOLD THAT CONSTITUTES THEM, INTO AERIAL HYPHOMYCETE.

I had to carry out numerous and varied culture tests to obtain the hyphomycete forms of cancerous tissues; they were carried out on a series of eighteen cancers: thirteen breast cancers, four uterine cancers, one of which was complicated by a dermocyst, a cancer of the laryngeal region.

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The tumor and neighboring lymph nodes, immediately isolated by the surgeon, are placed and preserved in an aseptic container.

To obtain the aerial forms of the cancerous mold, the procedure is as follows:

The tumor and the largest of the lymph nodes are split in half. The section is scraped with a scalpel and the scraping is spread on agar tubes (at least a dozen). Six broth matras are also inoculated.

Culture can also be done by cutting thin slices about one square centimeter by one millimeter thick, which are spread on a dozen agar tubes and on carrot tubes.

All cultures are kept at *room temperature*, except three broth matras, which are kept in an incubator, two at 30°C, the other at 38°C

Cultures generally behave as follows:

1. Mold appears quickly (in four to fifteen days) on agar or carrot tubes.

2. A bacterial culture, often forming a mycelial veil that emits spore-bearing hyphae, develops in four to fifteen days in the flasks.

If no mold develops on the agar or carrot tubes, one of the bacterial broth cultures is inoculated onto agar tubes. The semi-liquid material that develops on these is then transferred to various solid media: meat, egg yolk, casein, etc., and even onto other agar tubes, where it generally ends up transforming into aerial mold.

Tumor pulp cultured on agar always gives rise, apart from aerial molds, to a semi-liquid bacterial culture that can transform into mold if transported to other culture media.

Mold always grows on either medium, but sometimes only after several months.

The essential thing for the success of all these operations is to keep the cultures at laboratory temperature, around 18 to 23°C.

By these methods, I succeeded in obtaining the mold constituting the eighteen cancers I studied, without a single exception.

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When I carried out at the end of this research, I did not yet know the process for quickly obtaining gonidial forms of molds by injecting animals and culturing their organs.

This process, in relation to cancerous tumors, consists of depositing a little of the tumor scraping product in two cubic centimeters of water, so as to have a suspicious liquid, and injecting it into a guinea pig. The animal always dies. Immediately after death (usually two or three weeks after the injection), the pulp of the lymph nodes of the spleen, liver, and lung are inoculated onto agar tubes. Three to five days later, one almost always obtains a proliferation of molds on the lung and the same mold on the liver and spleen.

The errors to which the process exposes and the means of avoiding them are explained elsewhere.

Several gonidial forms of cancerous mold are usually obtained; one can ensure that they are all related to this one by injecting them into the guinea pig to obtain their transformation into other forms. This process is described in the chapter: *Multiplicity of gonidial forms of molds.*

Two different species can be obtained from the same cancer; this happened in the cultures of breast cancer No. 2. In this case, there could be no doubt, because the mold obtained concurrently with the specific cancer mold (a *Penicilium*) was the grey *Monosterigmata* form of syphilis, a typical mold that is impossible to confuse with any other because of the shape of the fruiting bodies, the multitude of spirilia it carries, and the macroscopic appearance of the culture; but the experimenter must be warned that, in this case, other forms of the syphilitic mold, more difficult to distinguish, notably the *Penicilium* form, could have developed in the cultures.

The experimenter should also know that molds growing in the body (permanent or chronic mycoses of infectious diseases, vaccinia, smallpox, scarlet fever, diphtheria, etc.) can exist in cancerous tissues and develop in cultures.

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Description of the anatomical elements constituting cancerous tissue. Their identification with the elements of molds vegetating in vitro.

General description of the anatomical elements of cancerous tissue.

I will present the results of this study by first reviewing, in a general way, the shapes of the anatomical elements of cancers; I will then describe the shapes observed for each tumor, particularly in a certain number of breast and uterine cancers.

For this study, we proceed as follows:

A clean section of the tumor is made, then the surface of the section is gently scraped with the blade of a scalpel; a thick liquid is thus obtained, a small portion of which is mixed with a drop of water previously placed on a slide; this drop is spread over the entire surface of the slide, fixed by heat on a heated plate, then hot-stained for five minutes on this plate with dahlia.

For a second examination, the surface of the section is scraped to remove the softer parts of the tissue, and when the fibrous tissue appears on the section, it is scraped off for examination as above.

In the study of eighteen cancerous tumors, made using preparations of this type, I observed that the cancerous tissue is constituted and characterized by the presence of mycelium, sclerotia, and fruiting bodies of various forms of mold.

Mycelium. Its presence is noted in different forms in the preparations:

1. It often takes the form of large continuous cylinders or finer filaments, without segmentation (Fig. 1, 2, 3, pl. 184), swollen in places and containing within them, at the level of these swells, large sporiferous masses, oval or spherical. The preparations show a large number of these sporiferous masses, oval, more or less spherical, irregular and freed from the mycelium, either naturally or by scraping the tumor.

We can also observe, either lateral chlamydospores (1, 2, 4, **4,(?)** fig. 1, pl. 184; 8, fig. 1, pl. 188), or terminal chlamydospores (17, 18, fig. 1, pl. 185) identical to those present in molds cultivated in vitro on liquid media.

2. Other times it appears in the form of large, very convoluted, flexuous branches, made up of distinct sections placed end to end. Some of these sections are long (length equal to four or ten times the thickness) and have the appearance of spindles or sausages, straight, arched, or even curved in an S or U shape (7, 8, fig. 1, pl. 184; 9, fig. 1, pl. 185). Other short sections are irregularly oval or spherical. All these sections are loosely connected to each other. Scraping the tumor detaches them from each other, and they are found separated in the preparations. However, sometimes four or five sections are observed placed end to end.

These mycelial articles are the characteristic elements of spindle cell sarcoma. I was able to transform the anatomical elements of the fetus into cultures that perpetuate themselves like bacterial cultures and *that contain these mycelial elements characteristic of sarcoma.* These elements are represented in plate 200.

3. In the tumors, we find fragments, sometimes very long, of large mycelial branches that have retained a shape identical to that of the large filaments of in vitro mold cultures. The segments are long and their articulations have retained their usual knobby shape (6, fig. 1, pl. 188).

4. Here and there, in cancer preparations, we often observe mycelial filaments presenting a series of successive dilations identical to those found, for example, in in vitro cultures of Penicilium on liquid media (11, 14, fig. 1, pl. 184; 11, 15, fig. 1, pl. 185; 1, 3, pl. 195).

5. We also sometimes see, in the preparations, fine, septate mycelial filaments, which exist identically in the in vitro aerial culture of the cancerous hyphomycete. We often find these filaments in the process of segmentation and, *if we look carefully, we find the isolated bacillary forms corresponding to the segments.*

Some of these filaments are poor in basophilic protoplasm and are difficult to stain with dahlia; others are dark blue, that is, are very rich in basophilic protoplasm; some of these are in the process of segmentation; this releases bacilli which turn strongly

blue by the dahlia and found scattered in the preparations. It was the study of a bacillary segmentation of this kind, existing parallel to a segmentation in the form of an acidophilic coccobacillus, which led me to the knowledge and isolation of the first cancer-causing hyphomycete.

Sclerotia. In all cancers, the presence of sclerotia is observed, often enormous, giving rise to large, flexuous and highly branched mycelial trunks (1, fig. 1, pl. 188; 8, 9, fig. 1, pl. 194).

Fructifications. Cancerous tumors contain a large mass of fruiting bodies of various shapes.

Sometimes, but rarely, certain fruiting bodies retain a shape similar to the sterigmata of *Penicilium* and which allows them to be identified.

Other fruiting bodies are easy to identify, because they are sporiferous hyphae (16, fig. 1, pl. 185; 7, pl. 195; 5, fig. 1, pl. 196) identical to those found in in vitro bacterial cultures or to those of dermatophytes.

But, in general, the fruiting bodies of molds are so deformed in cancer that, to recognize them, I had to conduct extensive research on their development in various environments. Multiple observations combined finally allowed me to follow all the fruiting forms step by step until their perfect development.

The fruiting bodies of the mold constituting a cancerous tumor mainly present the following forms:

A. The shape of very elongated fusiform organs, pointed at both ends, placed at the end of a long, slender peduncle. They are homologous to the spindles described by Sabouraut in the hanging drop dermatophyte cultures (10, 12, fig. 1, pl. 185; 2, fig. 1 pl. 188).

B. The shape of the pectinate or crooked organs, described by Sabouraut in the dermatophyte cultures (9', fig. 1, pl. 188).

C. The shape of globular, spherical, or rounded and irregular organs, oval, pear-shaped, bean-shaped, etc. (3, 16, 17, fig. 1; 1, fig. 2, pl. 184; 17, 18, pl. 185).

The fruiting bodies of forms A and B are the tips of spore-bearing hyphae of *Penicilium* that do not reach full development. They are the equivalent of a fruiting tip of *Penicilium* whose ramifications, as well as the sterigmata that terminate them, remain fused.

We also note in preparations of cancerous tissue:

1. Granular masses having a size equal to or greater than that of a white blood cell, rich in basophilic protoplasm and in the process of germination, that is to say, emitting branched mycelial filaments (2, fig. 2, pl. 190).

2. Masses of oval agglomerations colored very dark blue by the dahlia, and appearing to open at one end to reject outside a mass of spores and especially zymospores (4, fig. 2, pl. 190).

Identification of the fruiting forms existing in cancerous tissues with the fruiting bodies of hypnomycetes.

In cultures of many species of *Penicilium* on liquid media or in their cultures on solid media, developed in the semi-liquid form, a globular mass in the shape of a sphere or pear is often formed at the end of the hyphae or sporiferous branches, in which the sporiferous ramifications and the sterigmata develop (Fig. 1 to 5, pl. 21); sometimes, this globular mass is very elongated, even segmented, and takes the form of the fructifications described for *Epidermophyton inguinale* or for *Ackorion gypscum* (Fig. 1, 2 and 3).

After coloring by the dahlia, the ramifications and sterigmas in the process of forming can often be seen through the semi-transparent spherical mass (Figs. 1 to 5, pl. 21). Once these have formed, the wall of the sac detaches and falls off in the form of a vesicle or a thin-walled cap.

In certain species cultivated in vitro, the tendency of the fruiting end to branch is indicated by the appearance, on the terminal swelling, of several very visible points or projections. The projections noted on the external curvature of the pectinate organs of dermatophites have this significance.

We have seen elsewhere that the same mold develops in different forms described until now as distinct species, but which, in reality, are only different fruiting types.

Penicilium can develop in a simple form comprising a spore-bearing stem ending in a single group of sterigmata (*Citromyces* type). Often, this group of sterigmata does not reach full development. A spherical or oval ball terminates the gonidiophore (Fig. 7, pl. 21).

Diphtheria Penicilium often develops in this form in the false membrane; when the latter is stained with dahlia, globular masses, pedunculated or not, are often observed, colored pale blue, and *inside which the fully formed sterigmata of Citromyces are seen.*

Other fruiting forms such as sporiferous hyphae, terminal or lateral spores, and chlamydospores, are identified as fruiting bodies of molds, either solely by their characteristic shape or by their direct relationship with mycelial filaments in the process of segmenting.

Observation of the anatomical elements of the cancerous tissue in each tumor in particular.

The description of the anatomical elements is made here only for certain tumors taken as a type and mainly for two uterine cancers; it is made for four breast cancers in order to show the particularities presented by certain tumors. For others, I have limited myself to the description and representation of the specific mold of the cancer.

Uterine Cancer. Cancer No. 1.

Large, hard, fibrous, and severely ulcerated cancerous uterus. The parts used for microscopic examination and cultures are the furthest from the ulcerated region. Of course, all precautions were taken (this said once and for all and applying to the following tumors) to ensure that the tissues removed were preserved from any contamination.

One of the gonidial forms of the cancerous mold was obtained from the tumor itself, which had spontaneously molded in the icebox, and also from a fragment of aseptic tumor preserved in a bottle. It is a mold of the *Sterigmatocystis* type.

Plates 184, 185, 186, and Figure 1 of Plate 187 represent the constituent elements of the tumor observed in the scraping fluid. Figures 1, Plate 184, and 1, Plate 185, are photographs of drawings with an approximate magnification of 650.

At the time I made my first observations, I was not yet able to photograph my preparations. Figures 2, 3, plate 184; 2, 3, plate 185; 2, 3, plate 186, are photographs at a mag-

nification of 650 diam. of cancerous elements in my preparations; Figures 1, plate 186, and 1, plate 187 are photographs at a magnification of 235. These photographs were taken one year after the original drawings.

This tumor is the one that allowed me, most easily, to identify the cancerous elements with those of the molds. Indeed, one will see in Figure 1, plate 184, portions of branched (1, 2) and segmented mycelium bearing chlamydospores (4) that are easy to identify.

The tumor contained a large quantity of granular bodies (3) of generally oval, irregular shape, which are also found in large numbers in cancers and which are, as a rule, strongly basophilic. I easily recognized that these oval granular bodies were formations developed in the interior of the mycelium of the mold and spontaneously expelled to the outside or detached by scraping (1, fig. 3, pl. 184; 1, fig. 1 and 1, fig. 3, pl. 185); some, among these oval bodies, are voluminous mycelial articles (5, fig. 1, pl. 184) detached from the mycelium by scraping.

Other granular bodies, very elongated, often spindle-shaped with obtuse ends (6,fig. 1, pl. 184), other times irregular or curved into a horseshoe (9, fig. 1, pl. 185; 1, fig. 3, pl. 186) have the same origin. These granular bodies are strongly colored by the dahlia.

Other granular bodies, elongated into spindles or curved into a V, are fragments of mycelium, some of which are made up of several articles (7, fig. 1, pl. 184), others of a single very elongated mycelial article (8, fig. 1, pl. 184). These spindle-shaped bodies are much less strongly colored by the dahlia than the previous ones.

Finally, we find a third type of spindle-shaped body, but whose ends are tapered and often extended by a long filament (9, fig. 1, pl. 184; 10, fig. 1, pl. 185). They most often contain a large granular nucleus (1, fig. 1, pl. 184); other times, they are stuffed with spores (10, fig. 1, pl. 184). By searching in the preparations, we easily see that these bodies are mycelial dilations; we sometimes find two or three following one another on the same mycelial filament (11, fig. 1, pl. 184; 13, fig. 3, pl. 185); they are identical to the normal fixed cells of connective tissue.

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As for the mycelium, it is found everywhere in the preparations and in the most diverse forms (1, 8, 11, fig. 1, pl. 184; 10, 11, 12, 13, 14, 15, fig. 1, pl. 185). Certain mycelial ramifications are terminated by tendrils similar to those found on the mycelium of certain *Trichophyton* and most *Aspergillus*.

In the preparations, we notice large masses, either pear-shaped (12, fig. 1, pl. 184), or ovoid or spherical (13, fig. 1, pl. 184; 1, fig. 2, pl. 184; 7, fig. 1, pl. 185), stained strongly dark blue by the dahlia; these are agglomeration masses or germinative masses; some emit large mycelial branches (sclerotia) (13, fig. 1, pl. 184). I believe that these large germinative masses are the cells that are designated, in cancer sections, by the name of giant cells. Other germinative masses in the process of formation (leukocytes, 18, fig. 1, pl. 185) are numerous in the preparations.

As fruiting bodies, I found in this cancer:

1. Spore-bearing hyphae bearing fine refractile spores that do not stain the dahlia (16, fig. 1, pl. 185).

2. Large terminal refractile spores, borne at the end of fine mycelial filaments (15, fig. 1, pl. 185).

3. Spherical granular bodies borne at the end of mycelial filaments (14, fig. 1, pl. 184; 17, fig. 1, pl. 185). Some of these terminal granular bodies have the same shape as in certain developing Penicilium and are surely the beginnings of the groups of sterigmata of the gonidial hyphae (16, 17, fig. 1, pl. 184); in one of them (16), the beginnings of branching are already visible.

4. Terminal ovoid granular bodies, surrounded by a spiral filament that can be stained in intense blue (18, fig. 1, pl. 185) and which I consider to be perithecia in the process of formation. I have found these fructifications in other tumors; they are quite rare in preparations.

An enormous mass of spherical granules, varying in size from 0.1 to 1 μm, is observed in the preparations; some are strongly stained with dahlia, others refractile and unstainable. The largest are spores. The smallest are zymospores. Microscopic examination of the tumor scraping fluid, stained with a little dahlia, allows their Brownian motion to be easily seen.

The tumor preparations also contained bacillary elements resulting from the segmentation of the fine mycelial ramifications. These will be seen by examining Figs. 2, 3, Plate 184; 2, 3, Plate 185; and 2, 3, Plate 186.

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By examining photographs 1, plate 186, and 1, plate 187 with a magnifying glass, most of the elements noted in this description can be found.

Uterine cancer no. 2. Large, hard, fibrous, ulcerated uterus.

The cancer-causing agent was obtained in the form of mold that developed spontaneously, on the one hand, on the uterus preserved in the refrigerator and, on the other hand, on an aseptic uterine fragment preserved in a flask at room temperature; it was also obtained by transforming the bacterial culture developed by inoculating tumor fragments into broth. The bacterial culture gives off the odor of methylamine. Transferred to agar and carrot, it gave rise to the cancer-causing *Penicillium*. This *Penicillium* is represented in photographs 2, 3, 4, 5, and 6, plate 188.

Figure 1, plate 188, is a photograph of a drawing representing the characteristic elements of this cancer. These various elements are shown at a magnification of 650, except for sclerotium 1 drawn at a magnification of 233. This sclerotium is a large germinative mass having emitted large, highly ramified mycelial branches which constitute the fibrous tissue of the cancer.

The preparations contain:

1. Fusiform masses (2, fig. 1, plate 188), or elongated sausage-shaped masses (3), and oval granular masses (4), which have the same origin as the identical masses described for the previous tumor.

2. Germinative masses (leukocytes) in the process of formation and in different states (5), some of them large.

3. Mycelial branches, some of which (6) have retained an appearance identical to that which they have in the hyphomycete preparations; tendril-like mycelial branches (7).

4. Pedunculated chlamydospores still adherent to mycelial branches (8).

5. Gonidial hyphae bearing at their ends the beginnings of the sterigmata. Some of these beginnings (9) already bear the clear trace of their division into groups of sterigmata.

Uterine cancer No. 3. Very remarkable tumor formed:

1. By a spherical dermal cyst about 12 centimeters in diameter, ossified, adherent to the body of the uterus.

The cyst was formed by a large number of irregular bone fragments, connected together by excessively tenacious fibrous tissue, some bearing hairs.

2. By two distinct fibromatous masses, the size of a small tangerine and adherent to the body of the uterus.

3. By a fibromatous uterus.

The fibrous tissue of the dermoid cyst was cultured. It gave rise directly to the mold and to a bacterial culture broth which, transformed into a hyphomycete by transfer to solid media, reproduced the same mold.

I first obtained, on the tissue of the cyst preserved in the icehouse, a rudimentary form (Figs. 1, 2, 3, pl. 189) of the mold; it was, as shown in figure 1, plate 189, a *Sporothricum*. But, on careful examination of this, I found gonidial hyphae of *Penicillium* (1, fig. 2, and 2, fig. 3, pl. 189) in the process of development and still rudimentary, among the fructi-

fications of *Sporothricum.* This culture of hyphomycetes contained fructifications (fruiting bodies) (1, fig. 3) identical to those which have been described and figured in the two previous tumors under the name of terminal chlamydospores.

Let us note here that this *Sporothricum* type, represented in Figure 1, has an identical appearance to the organic mold constituting the medullary pia mater and certain regions of the nerve centers; there is here, not only an apparent identity, but a real identity, the vegetative form being the same and the origin common: it is the same form of the human organic mold observed under different conditions.

The perfect *Penicilium* developed directly on tumor fragments cultivated on carrots at laboratory temperature. It is represented in photographs 4, 5, 6, 7, 8, and 9, at a magnification of 650. The culture of *Sporothricum* or rudimentary *Penicilium* is straw-colored; the culture of perfect *Penicilium* is dark green. Subsequently, the direct transformation of *Sporothricum* into the perfect *Penicilium* form was observed in one of the cultures.

Uterine cancer No. 4. Quite large, ulcerated uterus.

Culture of the cancerous tissue and the uterus preserved in the icebox gave rise to the cancerous mold, which was a *Penicilium*. This *Penicilium*, whose development is very instructive, has gonidial stalks ending in sacs in which the groups of sterigmata develop. These fruiting bodies are shown in Plate 21 (Figs. 1 to 5), and have been described previously.

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Breast Cancer

I cultured thirteen breast cancers, all large and already spread to the lymph nodes. In my microscopic examinations of the tumor elements, I limited myself to noting the identity of their forms and origin with the elements already shown for uterine cancers; the abundance of figures in this work limiting the possibility of showing them all, I will only mention here certain elements encountered in two of the most remarkable forms.

For others, I focused on searching, in the tumor preparations, for fragments of the cancer-causing agent allowing the observation of its vegetative form in order to compare it with that of its gonidial forms cultivated in vitro. Photographs collected for this purpose can be found on plates 190, 191, 192, and 193.

As for the culture of the cancer-causing agents, it was obtained for the thirteen cancers studied.

Breast Cancer No. 1.

Photographs 1, plate 190, and 1, plate 191, represent tumor fragments contained in a preparation of the liquid obtained by scraping the tumor. Examination of this preparation showed that the tumor was composed of mycelial arborizations covered with an enormous mass of fruiting bodies identical to those of the mold obtained in vitro (Fig. 2, plate 191). These fruiting bodies, colored by the dahlia, took on the same very distinctive bright blue coloration in both cases. As in the in vitro culture, the mycelial ramifications of the cancerous mold terminated in compact panicles of fruiting bodies (1, fig. 1, plate 191). The examination of the tumor and the mold (2, pl. 191) could not leave the slightest doubt that the latter was vegetating in the tumor in the very form contained in figure 2 of plate 191.

Figure 2, plate 190, is the photograph of a drawing at an approximate magnification of 650 D, representing germinative masses (1, 2) in the process of germination; two of them (1, 3) emit mycelial filaments bearing spindle-shaped dilations (5, 6); one of them, open (4) lets escape a large mass of spores and zymospores agglomerated by a

gelatinous substance; it is probable that this emission of spores is not the normal role of this agglomeration mass, and that it only releases its contents because it has been traumatized by the scraping of the tumor.

The culture of the cancerous tissue gave rise to three different gonidial forms of molds. Two of these forms related to the cancer-causing agent, the third was syphilis.

Of the first two molds, one (Fig. 2, pl. 191) is a normal gonidial form of human organic mold; it is a mold with fairly long, loosely packed hairs, greyish-cream in color; the other is the *Penicilium* form of human organic mold; it is green in color (Figs. 1, 2, 3, pl. 192).

As for the syphilis culture (Fig. 4, pl. 192), it was of the *Monosterigmata* type and presented its characteristic appearance: a prominent, strongly mammillary, mouse-grey, short-haired, and very slow-growing culture. Beneath the culture flowed a semi-liquid material of reddish-pink color, a frequent but not constant characteristic of cultures of the gray *Monosterigmata* form of syphilis.

The cancer patient was therefore syphilitic. The influence of the presence of the syphilitic mold on the development of breast cancer remains problematic.

Breast Cancer No. 2.

I obtained the mold constituting this cancer in the form *Acremonium alternatum* (Figs. 1 and 2, pl. 193).

Examination of preparations of the tumor scraping fluid showed that it was made up of large bundles of mycelial filaments, like the mold cultivated in vitro (compare Figs. 4, tumor, and 1, mold in vitro). We also note, on the edge of these bundles(Fig. 4), the existence of gonidiophores similar to those of the mold (Fig. 1) cultivated in vitro.

On the other hand, by examining Figure 3 (tumor), we observe the presence of oval gonidia identical, in shape and dimensions, to those of the mold of the *Acremonium* alternatum type (Fig. 1).

It is therefore probable that the cancerous mold vegetated in the tumor in this *Acremonium* form.

Note that this same gonidial form is obtained identically by transformation of cultures of human tuberculosis, which are, themselves, a vegetative form of the human organic mold.

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Breast Cancer No. 3.

This cancer had the peculiarity of constituting a mass hard as cartilage, difficult to cut with a knife. In a scraping preparation, it presented the usual elements of cancers, some of which are represented (1, 2, 3, 4, 5, 6, 7) in Figure 1, Plate 194.

The preparations contained leukocytes (1, Fig. 1), germinal masses in the process of formation; oval intramycelial nuclei (2); fusiform mycelial dilations (3, 7) (fixed cells of the connective tissue); dilations of the same kind emitting mycelial ramifications (6); terminal fruiting masses stuffed with spores (4), etc.

These various elements constituted only a small part of the tumor; it was mainly made up of enormous sclerotia emitting large mycelial trunks branching on all sides and intertwined, which gave the tumor its resistance to cutting and its cartilaginous consistency. Figure 1, plate 194, represents (8, 9) two of these sclerotia emitting their branched mycelial trunks.

This cultured cancerous tissue gave rise to the *Penicilium* shown in photographs 2, 3, 4, 5, 6, of plate 194. Its culture is green.

Breast cancer No. 4.

This large cancer had the particularity of being very fatty. Scraping the hardest parts, consisting of pure cancerous tissue and which appeared free of fat, yielded a liquid heavily laden with oily droplets.

Microscopic examination of this cancer revealed some interesting features. The mold appeared in the form of large mycelial filaments bearing swellings (1, fig. 1, pl. 195) similar to those seen on the mycelial filaments of certain *Penicilium* vegetating on liquid media, and other more elongated, enormous swellings, in irregular spindles. Examination of these large spindle-shaped dilations, one of which, typical, is represented in plate 195 (2), shows that they are identical to the mycelial dilations which constitute the fixed cells of the connective tissue; this dilation emits mycelial branches 4, 5, 6, as do many fixed cells. Certain ramifications bear sporiferous hyphae (7, 8); others bear a series of small dilations.

Large dilations are stuffed with highly refractile granulations of various sizes; torn at several points (9) by manipulations, they release these granulations (10) which are fat globules.

This cancer was therefore characterized, on microscopic examination, by the presence of a considerable number of enormous mycelial dilations packed with fat globules, a fact which corresponds to the fatty appearance of the tumor.

Apart from the mycelial dilations, figure 2, plate 196, shows the presence, in the cancerous tumor:

1. Long mycelial filaments (1) bearing highly branched sporiferous hyphae (2).

2. Lateral chlamydospores (11, fig. 1, pl. 195; 3, fig. 2, pl. 196).

3. Long mycelial filaments appearing flattened (4), ribbon-like, wide, undulating, one of the edges of which, strongly stainable with dahlia, is made up of a continuous basophilic filament (6). These filaments end in a globular body around which the basophilic filament (6) spirals. These bodies, already noted in uterine cancer No. 1, plate 185, appear to be fruiting bodies (Früchtkörper) in the process of formation.

Apart from these elements, the preparations contained the other usual elements of cancers. Culture of the cancerous tissue produced the *Penicilium* shown in photographs 2, 3, 4, 5, 6, 7, plate 196.

For all the following cancers, I have limited myself to providing a photographic representation of the cancer-causing mold obtained by direct culture of the cancerous tissue. Here is an indication of the figures representing these agents:

Breast Cancer No. 5. The mold constituting this cancer (Plate 197) provides an example of the variability of gonidial forms in the same culture, as shown by fruiting bodies 3, Figure 1; 4, Figure 2; 2, Figure 3; 1, Figure 4.

Breast Cancer No. 6. A rapidly evolving tumor, existing simultaneously with another identical tumor in the nape of the neck, determined to be a sarcoma by histological examination carried out at the hospital.

The culture gave rise to the *Penicilium* represented in photographs 1, 2, 3, Plate 198.

Breast Cancer No. 7. Its culture gave rise to the *Penicilium* represented by photographs 4, 5, 6, 7, 8, 9, 10, 11, plate 198.

Breast Cancer No. 8. The cancer-causing agent was the *Penicilium* represented in Figs. 1, 2, plate 199.

Breast Cancer No. 9. This is the first of the cancers I examined and was the star- ting point for all the research contained in this work. I first obtained a bacterial culture as indicated at the beginning of this work. The cancerous *Penicilium,* represented by photographs 3, 4, and 5, plate 199, was obtained by transferring the bacterial culture to solid media: egg yolk, meat, casein, and agar. I would like to remind you that this was a most dangerous cancer, with rapid progression; the volume of the lymph nodes in the groin had already reached the size of an orange, while the volume of the tumor did not yet exceed that of a mandarin.

Breast Cancer No. 10. The cancerous mold is represented in Figure 7, plate 199. I did not push its transformation to the *Penicilium* type.

Finally, Figure 6, Plate 199, shows a photograph of the cancer-causing agent of a tumor (recurrence of a previous tumor already operated on) in the larynx region. The mold obtained is identical to that in Plate 191. It is a *Penicilium* arrested in its development.

For other breast tumors that I have not photographed, for a tumor of the thyroid body, the pathogenic agent that I isolated is a *Penicilium* identical to the previous ones, and the representation of which would add nothing to my demonstrations.

Identification of the mold constituting cancerous tumors in humans with the specific organic mold that constitutes their organism.

This identification results from the comparison of the gonidial forms of molds obtained by culturing cancers with the gonidial forms obtained by culturing normal fetal tissues;

the latter are shown in Plates 174, 175, and 176. This comparison of the gonidial forms demonstrates:

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1. That the *Penicilium* and *Citromyces* forms obtained by culturing tumors are identical to the *Penicilium* and *Citromyces* forms obtained by culturing normal fetal tissues and at the same time identical to the *Penicilium* and *Citromyces* forms obtained by transforming tuberculosis cultures.

2. That the *Sterigmatocystis* form obtained by culturing uterine cancer No. 1 (Figs. 2 to 6, pl. 187) is identical to the *Sterigmatocystis* form of the specific human organic mold.

The distinction between different *Sterigmatocystis* forms is very difficult and must be completed and confirmed by transforming the cultures into various gonidial forms.

3. That the very particular gonidial form obtained by culture of breast cancer No. 1(Fig. 2, pl. 191) and by culture of a laryngeal cancer (Fig. 6, pl. 199) is found to be identical for the organic mold specific to humans (Figs. 9, 11, 12, pl. 176).

4. That the *Sporothricum* form obtained by culture of uterine cancer No. 3 (Figs. 1, 2, 3, pl. 189) is identical to the *Sporothricum* form of the organic mold specific to humans, since they both gave rise to the same *Penicilium* form.

In addition to these gonidial forms, four others were obtained:

1. An evolving mold whose type was not fixed (Pl. 197, breast cancer no. 5) and which presented fruiting bodies of *Aspergillus* (3, fig. 1; 1, fig. 4), fruiting bodies of *Sporothricum* (4, fig. 2) and fruiting bodies evolving to the *Penicilium* type (2, fig. 5). We can identify this mold by comparing its gonidial hyphae of *Sporothricum* (4, fig. 2, pl. 197) with those of the two molds represented in figures 6, plate 199 and 2, plate 191, which are forms of the organic mold of man For these three molds, it is the same original form that evolves in plate 197 into the *Penicilium, Sterigmatocystis,* or *Aspergillus* forms.

2. A mold affecting a gonidial type that I have observed several times (Fig. 1, plate 193, breast cancer no. 2); this form is indeed related to the organic mold of man, because I obtained it from the caseous matter of the tuberculized lung (Fig. 15, 16, plate 173).

There can be no doubt that this gonidial form indeed belongs to the cancerous mold, because it vegetated in this very form in the tumor.

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3. A mold of the Monosterigmata form, breast cancer no. 9. (Fig. 7, pl. 199). This mold is identical to the Monosterigmata form of the human organic mold because I obtained it by culturing the caseous matter of the human tuberculous lung (Fig. 13, 14, pl. 173).

4. A mold of the *Monosterigmata* type, foreign to the organism (breast cancer no. 1, fig. 4, pl. 192). This mold, whose culture is characteristic, was the syphilis mold; it vegetated concurrently with the mold constituting the cancer, which was isolated in the *Penicilium* form (Fig. 1, 2, 3, pl. 192).

From this presentation, it follows:

1. That, in all cases, identification could be made between the mold constituting the cancer and the human organic mold.

2. That the mold constituting cancers vegetates in a gonidial form different from the normal fruiting forms of organic mold. Proof was provided for breast cancers No. 1 and No. 2, in which the gonidial form could be observed and determined.

* * *

Let us now summarize the results of the observations made so far on cancerous tumors.

A first series of observations led me to conclude:

1. That cancer is constituted by a mold.

2. That the anatomical elements of cancer are those of the cancerous mold.

3. That the culture of cancerous tissue in vitro gives rise to the cancerous agent in the hyphomycete state.

These first conclusions, the results of observations limited to cancer, seemed to mean that it is always constituted by a mold, and that this mold, being heterogeneous, penetrates the organism through an infection from outside **(1)**.

The question of the nature of cancer, which thus appeared to be resolved, was, however, only incompletely resolved. Indeed, the full significance of my first observations could not have appeared because I was ignorant at that time of the nature of the tissues of the animal organism.

Subsequent research that established this nature added the following to the previous conclusions:

1. The mold whose vegetation constitutes the cancerous tissue is the specific organic mold of the individual, at least in the vast majority of cases.

(1) *A certain number of authors had already isolated a certain number of hyphomycetes from cancerous tumors, a fact which, moreover, had not settled the question of the nature of cancer.*

2. Perhaps some cancers are constituted by the simultaneous vegetation of two molds: a parasitic mold foreign to the organism, and the specific organic mold modified in its evolution.

3. Cancer results from a change in the normal vegetative form of the specific organic mold and its abnormal multiplication. The specific organic mold loses the organic type to resume the ancestral type or ordinary vegetative type of hyphomycetes, which no longer obeys the organic laws governing the development of tissues.

4. The abnormal multiplication of the organic mold probably comes from its return to the usual multiplication processes of hyphomycetes in in vitro culture, replacing the normal multiplication or growth processes of tissues. This last conclusion is supported by the presence, in cancers, of fruiting bodies characteristic of certain gonidial forms and identical to those presented by these forms in in vitro culture.

The shape of certain elements of cancerous mold is the same as that of the elements of normal organic mold, a form that can be observed in various organs. But cancerous mold is generally distinguished by the large size of its mycelial elements, by the considerable mass of its fruiting bodies, and by their abnormal type.

Experimental transformation of human organic mold into elements of cancerous tissue.

From the preceding demonstrations, it follows that cancerous tissue is indeed constituted by an abnormal vegetative form of normal human organic mold.

I was able to acquire the final, most complete proof of the accuracy of this conclusion by reproducing in full, with the normal elements of the organic mold of the human

fetus, the anatomical elements characteristic of the most serious form of cancer: globocellular sarcoma and spindle cell sarcoma. The demonstration is so complete that these elements, developing very rapidly, multiply by successive generations in in vitro culture, like bacterial cultures.

I obtained these elements in the following way:

Having taken fragments of organs from human fetuses dead at term, brain, lung, liver, spleen, I reduced them to a pulp by grinding them with a small spatula in a sterile watch glass. This pulp is spread on agar in tubes (in the upper half) which are stored at a temperature of 22-24°. After a certain time (four to fifteen days), a creamy substance develops in the lower part of the seeded region, which gradually extends to the bottom of the tube. A particle of this substance is inoculated into the broth, either in an ordinary flask or in a flask containing a layer of agar on the side, as explained on page 415;

in the latter case, the inoculation is not in the broth, but on the agar, one centimeter above the broth; the creamy substance, developing towards the lower part, spreads over the broth in the form of a surface veil. But, often, this veil is formed even by inoculating directly into the broth.

By this method, I obtained, from the culture of the spleen pulp of a fetus that died at term, a veil culture on broth formed by elements typical of spindle-cell sarcoma. The veil is thin and made up of mycelial elements fragilely joined together; they disintegrate with the greatest ease when the veil is transferred to a slide for preparation. It is therefore advisable to remove it carefully, place it flat on the slide in a few drops of water, and gently dissociate it. Under these conditions, fairly long, entire portions of mycelium remain.

Different regions of this veil are represented in Figures 1 to 8 of Plate 200. It is clearly visible in Figures 1, 3, and 8 that these elongated, spindle cell, sausage shaped elements , etc., are the segmental elements that constitute the mycelium. Other intercalated elements are short, ovoid, or nearly spherical.

Identifying these elements is easy by comparing them to those contained in a preparation of the pulp obtained by scraping a cancer section with a scalpel; this comparison, made with the elements of cancerous tissue contained in plate 186 (photo graphs of the elements of a uterine cancer), is most suggestive: the elements of uterine cancer are found, identical, in the in vitro culture. This comparison, made by the reader, under a magnifying glass, will inform him better than any description.

The culture reproduces from generation to generation without difficulty. A small portion of the veil is transferred to the surface of a matrass of ordinary glycosed peptone veal broth; after three or four days, the veil has invaded the entire surface of the broth; reproduction took place in my cultures without change of shape; on ageing, the veil falls to the bottom of the matrass; a portion of the deposit inoculated in new broth reconstituted a veil.

I only took organ samples from three fetuses; each of them allowed me to obtain a veil culture, one of which has just been described. In the other two cases, the culture, obtained with the pulp of the lung tissue, contained almost exclusively globo-cellular elements. These cultures have already been described on page 415; photographs of the veil elements are contained in plates 179 and 180. The reproduction of these cultures was as easy as that of the previous one and their growth as rapid. The elements contained in the veils are typical of globocellular sarcoma.

It has been demonstrated in the study of tuberculosis (page 419) that inoculating the guinea pig with the globocellular elements of these latter cultures causes tuberculosis.

This fact does not imply that they are tubercular elements and not cancerous elements. They possess both qualities. In reality, there is no essential difference between cancerous matter and tuberculous matter. Both come from the specific organic mold, and they are both only forms of it.

Indeed, it has just been demonstrated that it is possible to obtain, in vitro, the transformation of the elements of the normal organic mold into a culture of elements identical to the cancerous elements, the inoculation of which confers tuberculosis on the guinea pig.

It has also been demonstrated that broth culture of cancerous tissue can give rise to a bacterial culture consisting of basophilic bacilli and coccobacilli.

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It has finally been demonstrated elsewhere that the caseous matter of tubers can undergo a fundamental change in form (represented in Plate 181) which is accompanied by the formation of basophilic bacilli, coccobacilli identical to the previous ones, and even streptococci, whose *immediate origin is visible.*

It appears possible to transform the caseous matter of tubers into elements identical to the cancerous elements; this transformation is currently being researched.

Mode of propagation of cancer to neighboring organs.

By what elements does cancer spread to the lymph nodes and other organs? By the same elements that propagate the infection for all pathogenic germs.

It is the zymospores that carry out this transmission, either by themselves or by carrying the mold spores, thanks to their Brownian motion, through the spaces of the connective tissue to the lymph nodes and beyond. Transported far away, the spores and zymospores agglomerate into germinative masses that constitute new cancerous foci and determine the generalization of the cancer. It is probably these spores and zymospores of the cancerous mold that various authors have mistaken for cocci and described as the pathogenic agent of cancer.

The subtlety of zymospores, their mobility, which they owe to their Brownian motion and which allows them to pass everywhere, even through porcelain filters, explain the early danger of propagation of cancerous tumors and their generalization; this nature of the propagating elements of cancer justifies the conclusions, the only effective ones, which surgeons have reached to date:

1. Ablation as early as possible.
2. Ablation as wide as possible.

We also realize the impossibility of judging whether, even in the case of early and very wide ablation, the zymospores or other multiplying elements of the cancerous tissue have not already exceeded the limit of the extirpated region.

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Etiology of Cancer.

We now know the nature of cancer:

It consists of the growth of an abnormal form of specific organic mold. While the research presented so far tells us about this nature and explains the spread of cancer to the lymph nodes and other organs by the spores and zymospores of the cancerous mold, it does not provide us with precise information on the cause of its development.

However, the knowledge acquired in the research presented in this work informs us about the conditions that can determine a deviation of organic mold. These conditions are:

1. The formation, through trauma or any other cause, of a blood effusion in which white and red blood cells can form large germinative masses, becoming the origin of independent mycelial vegetation.

Such an effusion can play such a role even more easily if it contains elements of neighboring tissues and disorganized connective tissue. It can be predicted that such an effusion can give rise either to the formation of a bacterial culture, that is, an abscess or phlegmon, or to the formation of a directly mycelial culture, that is, the formation of a tumor.

2. The separation of a group of anatomical elements from their normal connections by trauma or any other cause.

The connections of these elements being eliminated and their nutrition modified, their vegetation has a natural tendency to resume the type of ancestral mold; this can evolve by forming a purulent collection; it is possible that it can evolve in the mycelial form by constituting a cancerous tumor.

3. Cancer is reproduced experimentally, either by prolonged application of X-rays (cancer of radiologists), or by application of tar to the skin (cancer of tar in mice).

It appears here that the influences which tend to destroy the specific *organic* mold first bring about the destruction of its organic properties. It seems certain, at least for X-rays, that organic mold, as soon as it is deeply affected, loses its organic type and vegetates in another form which causes ulcerations in the places where it develops.

This mode of production of cancer indicates to us that it can result from any cause which places the anatomical elements in a state of prolonged suffering and alters the organic properties of the specific mold which constitutes them; by losing its organic properties, the mold takes on *the free* vegetation of the ancestral mold, in very diverse forms. It has been demonstrated, by the in vitro culture of the organic mold of the fetus, that one of these vegetative forms is that of the elements constituting sarcoma.

4. It has seemed, until now, that cancer develops especially in organs subjected for a long time to a cause of continuous irritation (prolonged metritis for cancer of the uterus) or formerly subjected to a cause of acute irritation (mastitis for breast cancer). It is possible that this cause could be that of the deviation of the organic mold.

Another possibility may also exist: the presence, in the cancerous organ, of a heterogeneous mold causing a permanent mycosis and having previously caused an acute illness: measles, scarlet fever, smallpox (and, consequently, cowpox), diphtheria, typhus fever, etc.

I have indeed observed, in a breast cancer, the presence of syphilitic mold. In this case, the influence of this mold remains hypothetical; but we can nevertheless admit that the heterogeneous mold, vegetating at the expense of the anatomical elements, destroys their organization, but not the ancestral mold which, from that moment on, takes on another vegetative type: the cancerous type.

Cancer of the tongue is linked to syphilis, which, it seems, is always the original cause. It will only be possible to know the role played in this case by the syphilitic mold when it has been determined whether the cancerous tissue itself is constituted by the specific organic mold, or by the syphilitic mold, a difficult determination, because the latter, being the organic mold of the monkey, can take on an identical appearance to the organic mold of man.

However, this initial cause of cancer, which perhaps acts in certain cases and remains hypothetical, is probably not general. Indeed, in the very numerous experiments researching heterogeneous molds that I have carried out on animal organs, I have very rarely failed (almost never) to obtain in cultures the inoculated mold.

If heterogeneous molds had existed in the eighteen cancerous tumors I cultivated, I would likely have obtained and identified them. Having observed only the syphilitic mold in one case and the tetanus mold in another, I must currently conclude that the cancer consists of the organic mold alone.

Moreover, the fact that a piece of fetal spleen, reduced to a pulp and cultured on agar, then in broth, spontaneously gives rise to a culture of the characteristic elements of sarcoma, clearly demonstrates that the organic mold of man can *spontaneously* evolve into the cancerous form when placed in abnormal growing conditions. Trauma can therefore create these conditions by depriving a mass of elements of their nerve connections and their normal nutritive materials.

The action of X-rays, the known determining cause of cancer to radiographers, fully confirms this conclusion.

There is no need to invoke trauma or physical action to explain the development of cancerous mold. Indeed, it has been demonstrated that tubercular mold and cancerous mold have the same origin. However, tubercular mold develops spontaneously without any trauma. It seems obvious that the same can be true for cancerous mold.

It therefore follows from this presentation that a heterogeneous mold could act as a determining cause, like trauma, like X-rays, but that it does not constitute an element of the cancer virus. The latter is made up of organic mold only, and once its first elements have developed, its virus is made up of its elements alone, its zymospores, in particular.

Doctors Gye and Barnard recently announced that they had isolated the cancer virus; according to them, cancer would be caused not by one, but by two viruses, one specific, the other non-specific. The publications of these authors are not sufficient to judge the question; but it is possible, based on the determinations made in this work, to establish certain points. Indeed:

1. The characteristic elements of sarcoma can be obtained in stable, pure culture, by direct transformation of the fetal organ mold (transformation of the anatomical elements of the spleen and of the lung) cultivated in vitro on agar, it becomes certain, by this demonstration, that there is only one cancer virus and that this virus is constituted by the vegetative elements of the aberrant form of the organic mold which constitutes the cancerous tissue.

An identical transformation can therefore take place in the organism and, if it occurs, it is therefore the cancerous elements themselves which constitute the cancer virus.

2. The elements that, in breast cancer, cause the growth of cancerous tissue in the axillary and subclavicular lymph nodes, or even in more distant regions (armpit lymph nodes for a cancerous ulceration of the hand), are obviously those that constitute the cancer virus. Now, these elements are the zymospores (and perhaps larger bacterial elements mobilized by the zymospores) of the specific organic mold. In the lymph node, it is only this mold that is isolated. For most of the cancers studied, I have con-

trolled this fact and identified the cancerous mold of the lymph nodes with that of the initial tumor.

There is therefore only one cancerous virus, and this virus is made up of the zymospores of the aberrant and cancerous form of the human organic mold.

The fact that certain cancerous tumors, such as cancer of the tongue, are linked to syphilis does not detract from this conclusion; it is possible that the syphilitic mold, which is the organic mold of the monkey, can develop in the human organism, forming elements similar to those of cancer **(1)**.

It is also obvious that these elements, vegetating in this form, or even those of the usual form of the syphilitic mold (Monosterigmata form), can, by disorganization, cause the transformation of the organic mold into cancerous mold. But this is a very particular fact. The syphilitic cancer is an exception. It is certain that one can find, in a cancerous tumor, granulations or zymospores of the molds that cause vaccinia or smallpox, scarlet fever, typhus fever, etc., since the elements of the permanent mycoses of these diseases exist everywhere.

But the presence of these elements in cancerous tissue does not mean that they constitute an element of cancer or its virus, nor even that they exert a determining action on its development, as long as this demonstration has not been made.

If this demonstration were made, it would only mean that the vegetative elements of permanent mycoses constitute a cause of decay for the normal organic elements in contact with which they vegetate, as can other causes of a very different nature: trauma, X-rays, etc.; they would therefore be a determining cause of cancerous development, but not the cancerous virus itself.

From these facts, I will finally conclude:

1. That cancerous tissue is constituted by an abnormal vegetative form of the organic mold specific to humans.

2. That this abnormal form can be obtained, in vitro, in the very form of cancerous elements, of sarcoma for example, by culturing the anatomical elements, that is to say, of the organic mold of the fetus.

(1) *The formation of anatomical elements of a form analogous to those of cancer, by in vitro culture of the normal organic mold of the chimpanzee, is currently under study.*

3. That the cancer virus is constituted by the zymospores and bacterial elements of the cancer-causing mold, an abnormal form of the organic mold of man; that it is these zymospores and bacterial elements alone which cause the transmission of cancer to distant lymph nodes or organs.

The cancer virus is therefore not a heterogeneous virus. It is an autogenous virus, although it can develop the disease by inoculation into other individuals of the same species.

4. That cancer, linked to the evolution of syphilis, is a particular case which does not lead to a general conclusion relative to the usual development of cancer.

5. That the initial cause of cancer is often a decline of certain anatomical elements which can be caused by various causes: trauma, X-rays, persistent irritation (metritis), etc.

6. That cancer can, like tuberculosis, develop without the intervention of an external cause and result from general causes of decline: natural decline of anatomical elements in individuals through aging, physiological decline of certain organs at a specific time (menopause), decline caused by overwork and insufficient nutrition, etc.

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Is cancer inoculable?

It is very likely that if an emulsion of cancerous tissue were inoculated into the connective tissue of a healthy human, upstream of a lymph node, the latter would develop in the node. However, it is not certain that this will always be the case. Indeed, the inoculated cancerous tissue can undergo another evolution, notably pure bacterial evolution. It can give rise to another vegetative form, the tubercular form, for example.

What interests us most in this question is the possibility of reproducing cancer by inoculation in animals. This reproduction of cancer in animals is considered the absolute criterion for the quality or specificity of the virus isolated by experimentation. There is a conceptual error here, which is revealed by the facts presented so far. Indeed, when a guinea pig is inoculated with cancer pulp or an emulsion of elements derived from it, an operation is performed identical to that of inoculating pulp from the liver, spleen, or brain. It has been shown that inoculating the pulp of these organs first develops a bacterial culture in the animal's body, then, subsequently, a mycosis; it seems obvious here that the injection of this pulp will not, in this case, develop pulmonary, hepatic, or splenic tissue.

What reasons could lead one to believe that inoculating the pulp of cancerous tissue will develop it? There are none; on the contrary, there is every reason to believe that

this inoculation will cause the same effect as inoculating the pulp of other non-cancerous regions of the organism because, *in all cases, the inoculated product is returned to the state of the ancestral organic mold and even to its bacterial state.*

This fact is confirmed by the experiments described previously. Anatomical elements of the fetus being inoculated into the guinea pig cause it to become tubercular. The same result can be obtained by inoculation of cerebral substance.

But inoculating cancerous products from one individual *to another of the same species* can have a different effect, because the elements pass from one environment to another identical one. It is obviously for this reason that human cancer cannot be developed in a mouse, but that the tumor developed in another mouse can be reproduced in it.

The inoculability of human cancer to animals is therefore not a criterion for the specificity or quality of the cancer-causing virus.

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Is cancer contagious?

Since tuberculosis is contagious, there is no reason to believe that cancer is not also contagious. The elements of cancer transmission (spores, bacilli, and zymospores) are morphologically the same as the elements of tuberculosis transmission.

Due to the common origin of tuberculosis and cancer (the organic mold of humans) and the possibility of transformation of cancerous elements into tubercular elements or vice versa, it is to be expected that *contamination by cancerous products can cause tuberculosis, and that, conversely, contamination by tuberculous products can determine the development of cancer.*

This origin of certain cancers or certain cases of tuberculosis can only be established through observation and research into the causes of the development of these conditions in patients.

Cancer Treatment

We saw, at the beginning of this work, the guiding principles that guided me in my attempts to cure cancer. This research preceded all the observations that are the subject of this work. I was unaware, at that time, that the anatomical elements of cancer were mold elements. I thought, like many, that the cancer cell was an anatomical element of the animal organism that had acquired a very active power of multiplication. Since this multiplication obviously had to take place through the nuclei of cells, I thought that the

creation of antibodies capable of altering these nuclei could stop the development of cancer.

I was assisted, in these attempts to cure cancer, by Doctor E. Laval and Professor E. Desmaret.

The guiding principle of my first research was therefore to obtain anti-nuclear antibodies. To obtain them, I injected goats several times, two days apart, with a maceration of crushed cancerous tissue in water containing 1 part sodium carbonate; later, I added a maceration in distilled water, then a maceration in salt water at 7.5 parts per thousand.

The macerations were, before injection, filtered through sterile paper or mousselle. On the sixteenth and eighteenth days after the last injection, 200 to 300 cubic centimeters of venous blood were taken from which the serum was removed by centrifugation. The serum was then injected into the patients, starting with a dose of 1/10 of a cubic centimeter, and later continuing with doses of 2 cubic centimeters, 10 cubic centimeters, and 20 cubic centimeters, spaced every three days.

Believing that each patient had a specific cell, the serum injected was prepared with their cancer previously operated on. These attempts at cure were only carried out on cancer patients in an advanced state of development. The operations were performed by Professor Desmaret and the serum injections by Doctor Laval. Five patients were thus treated: three with breast cancer, two others with uterine cancer. Here are the results obtained:

Patient No. 1. Woman aged 40. Uterine cancer. Only the cervical region was removed, as total ablation had already become impossible. The removed region was used to prepare the macerations injected into a goat.

The serum was injected as stated above. The first injections did not cause any noticeable reaction, either local or general. From the fifth or sixth injection, a slight local reaction (redness) was observed, which subsequently increased. After subsequent injections, the skin became purplish over a width approximately equal to the palm of the hand, and an edema developed, disappearing after twenty-four hours. This reaction was due, not specifically to anti-cancer products, but to the repeated injection of the serum itself (Arthus phenomenon). The patient was thus treated for two months. The last injections caused general symptoms, facial congestion, tachycardia, which were, moreover, not very pronounced and very fleeting.

The patient lived for another year afterward, because the treatment was repeated on her, at that time, a second time.

The treatment did not appear to influence the development of the tumor because it continued to evolve, albeit slowly. But a more remarkable result was obtained. The patient, whose face was yellowish and cachectic, who had lost her appetite and her strength largely before the start of treatment, apparently regained a normal appearance. Her face became pink and colorful again, and her appetite and strength returned. A month later, the patient appeared transformed, which gave rise to hope for a favorable result with regard to the tumor. It perhaps evolved a little more slowly but continued to grow. A year later, the patient had become cachectic again. A new treatment had another favorable effect, although less pronounced than the first time. Since then, this patient has been lost to follow-up.

Patient No. 2. Woman aged 38. Uterine cancer
Total ablation is performed. The serum injections produce only an insignificant effect. Rapid recurrence and death after three months.

Patient No. 3. Woman aged 62, very obese. Breast cancer.
Total ablation of the breast cancer only. The tumor is already too generalized for the axillary lymph nodes to be removed.

The serum injections are given exactly as in patient No. 1 and produce the same symptoms; however, the general anaphylactic reactions are more intense at the last injections, although very short.

The action of these injections on cancerous cachexia was as favorable in this patient as in patient No. 1. The lymph node tumor in the axilla appears to have decreased slightly in size. The patient, who was arteriosclerotic, died of a cerebral hemorrhage after two and a half months of treatment, which was nearing its end.

Patient No. 4. 40-year-old woman. Breast cancer, large lymph node tumor of the axilla, supraclavicular lymph nodes.

At the beginning of treatment, the patient has a cachectic appearance. The injections are given as for other patients, but it is impossible to exceed the dose of 5 cubic centimeters of serum. It is even necessary to give injections not exceeding 1 to 3 cubic centimeters (diluted in 20 cubic centimeters of salt water) because of local and especially general anaphylactic reactions, which occur despite the slowness of the injections.

A powerful general action is observed from the third week of treatment. The cachectic appearance disappears completely, the face and mucous membranes become deeply colored again, and appetite and strength return. For four months, no increase in the size of the axillary lymph node tumor was observed, but an increase in the size and hardness of the supraclavicular lymph nodes was clearly perceived.

The patient, a domestic, was able to return to work and continued to do so for a year. She was lost to follow-up.

Patient No. 5. Fifty-year-old woman. Multiple-focal sarcoma of the breast and neck region. This is the cancer whose appearance is shown in photographs 1, 2, 3, plate 114. The treatment was applied by Doctor Valleteau of Mouillac. The injections had a favorable effect at first. They did not significantly delay the progression. The cachexia reappeared shortly after, and the patient died six months after the treatment.

We can therefore assess the effect of the treatment as follows:

1. The progression of the tumor is not stopped. It does not appear to be significantly influenced in some cases; in others, it appears somewhat delayed, without there being any certainty on this subject. Serotherapy treatment, at least under the conditions in which we practised it, is therefore incapable of stopping the progression of the cancer.

2. The treatment has a clear effect on the general condition of patients. When it is applied to patients already in a state of cachexia, it can suppress it, but only for a certain time.

What is the mechanism of this action?

It differs completely from the mechanism assumed in the guiding ideas presented above. Indeed, it is not an injection of nuclein from cancerous cells that is given to the animals to provoke the formation of antibodies. They are simply injected with the vegetative elements, spores, zymospores, and other bacterial elements, *and a certain quantity of the agglutinating hyaline matter of the cancerous mold.*

It has been stated previously that antibodies do not exist, at least not those whose action has been invoked so far. What develops in the serum of animals to which the elements of the cancerous mold are inoculated *is the agglutinating power* for these elements.

It is likely that it is only this agglutinating power that acts in cancer patients who are injected with animal serum prepared.

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This agglutinating power, which is conferred on patients, can, by agglutinating the spores and zymospores, prevent or delay their diffusion, that is to say delay the extension of the tumor.

In some cachectic cancer patients, the blood is found to contain an enormous quantity of zymospores and the serum is often pink, probably proof of the destruction of blood cells by these zymospores. The severity of the patient's condition can even be predic-

ted by assessing the quantity of zymospores in the blood. It is in those in the final cachexia period that the quantity of zymospores becomes the most considerable and the pinkish tint of the serum denotes the hemolysis of the blood cells. It is therefore conceivable that the injection of a product that agglutinates the zymospores, a state in which they lose their fermentative and hemolytic power, can temporarily eliminate the cachexia. It is likely that this is the mechanism of the only action that the serum of prepared animals exerts on the general condition of cancer patients.

We, thus, arrive at experimental confirmation of the conclusions we have already drawn previously. Serotherapeutic treatment can have an effect on the fermentative phenomena due to zymospores and on bacterial development. It has no effect against mycelial development. However, it is this which constitutes cancerous tumors.

Knowledge of the nature of cancer, a deviation from the vegetative form of organic mold, excludes a priori any attempt to destroy cancerous tissue by the action of physical agents or by injecting chemical substances into the body. It is obvious that the normal organic mold, that is, the individual, would be killed at the same time as the cancerous tissue.

The danger of the cancer spreading through the spores and zymospores of the modified organic mold *creates the need for very early surgery,* the only rational treatment.

As for future trials, the nature of the cancer indicates what they should aim for: *It is to return the organic mold, modified in its evolution, to its normal vegetative type.*

Only one procedure appears, so far, to have a clearly favorable effect in this direction; it is the prolonged and repeated application of heat, as high as possible, to the tumors (approximately 50°C). This treatment is unfortunately inapplicable to most tumors, especially the most serious ones.

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PNEUMONIA

The pneumococcus is considered the agent of pneumonia. The following facts show:

1. that it is probably not the agent, but only a witness to this disease;

2. that it is not a heterogeneous agent of infection, coming from the external environment, but a bacterial element *born spontaneously* in the human organism and resulting from its specific organic mold.

The viscous, thick, apricot-yellow mass, which constitutes the sputum of the pneumonic patient and which fills the alveoli and the fine bronchi of the pneumonic zone, contains, in addition to the pneumococci and leukocytes observed there, germinative

masses of various sizes in the process of germination and hyaline substance; the viscosity of the sputum is due to the latter; a large part of the mass of the sputum is made up of the germinative masses and the mycelium they emit.

Plate 201 shows photographs of sputum from pneumonia patients at a magnification of 245 diameters; the preparations in which these photographs were taken were made by crushing a piece of sputum between two slides, which were slid over each other, followed by heat fixation and dahlia staining. In addition to normal leukocytes, we see germinative masses emitting often very long mycelial filaments.

At the beginning of their development, these filaments are most often terminated by a dilation which affects the appearance of a trypanosome and which is indeed one; it has already been indicated, in fact, that *Trypanosoma gambiense* is constituted by a terminal dilation of a mycelial filament developed by a germinative mass of the pathogenic mold which is the agent of sleeping sickness (Plate 9).

The pneumococcus probably originates from the segmentation of some of these mycelial filaments.

The germinative masses of various shapes (round and oval) vary greatly in size (from 10 to 30 or more). In addition, a considerable mass of spores of various sizes and zymospores is observed in the preparations.

These various elements show that *the entire mass of sputum is made up of a culture of an evolving mold.*

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Indeed, if the gelatinous mass of pneumonia sputum is spread on sugar agar and the cultures are kept at a temperature of 20 to 24°, they become covered, from the third to the sixth day, with the mold that constitutes them. I observed only one mold, of the *Penicilium* type, on the cultures I made, and this mold was always the same in all cases of lobar pneumonia. It is shown in plate 202; figures 1, 2, and 3 relate to a case of frank pneumonia (soldier hospitalized at Val de Grâce); figures 4 and 5 to another case of pneumonia treated at home.

Examination of preparations of this *Penicilium* allows it to be easily identified either with the *Penicilium* form of the organic mold specific to man (Pl. 174 and 175), or with the *Penicilium* form obtained by transformation of cultures of human tuberculosis (Pl. 162 and 166), or with the *Penicilium* form obtained by culture of cancerous tissue (Pl. 198 and 199); it has been previously stated that the mold which constitutes tuberculous lesions or cancerous lesions is the organic mold specific to man, modified in its form and in its evolution.

The pneumonic lesion is therefore caused by the growth of an abnormal, pathological form of human organic mold; the initial cause that determines pneumonia is therefore the one that causes the transformation of normal organic mold into an abnormal form that vegetates independently, such as tubercular mold or cancerous mold, and like any heterogeneous mold.

This cause is likely, in most cases, as clinicians have long indicated, the action of cold, *cooling.* Cold is, in fact, one of the main causes that provokes the change in form of organic mold.

The initial cause of pneumonia is therefore not the pneumococcus and does not result from an infection by this agent, coming from outside. Pneumonia is spontaneous, idiopathic. The bacterial agent whose constant presence in the lesions, the pneumococcus, arises spontaneously in the lesion by mycelial segmentation of the organic mold and is only a witness, not a causal agent, of the condition. The agent, whose development causes the disorders of pneumonia, is the abnormal form of the modified organic mold.

These facts explain:

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1. That the pneumococcus frequently exists in the mouth and intestine of healthy people and is harmless there.

2. That pneumonia is not contagious, although a bacterial element constantly exists in pneumonic lesions.

3. That the presence of the pneumococcus is observed in many other primary human affections outside the coexistence of pneumonia, for example in pleurisy, pericarditis, endocarditis, keratitis, tonsillitis, peritonitis, etc., non-contagious affections. In these cases, as in pneumonia, the pneumococcus arises spontaneously in the organism and is a segmenting element of the abnormal organic mold vegetating in the lesions.

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BACTERIUM COLI

I studied the *coli* bacillus on two cultures of human origin provided to me by the bacteriology laboratory of the Faculty of Medicine.

I obtained its direct transformation into hyphomycete fairly quickly by inoculating it on meat, casein, and on slides of porous bodies (sterilized bone slide 3 millimeters thick, porous earth slide), kept simply moist by a few drops of glycosylated peptone beef broth placed in contact with the underside of the slides.

By this process, I only obtained the transformation in the gonidial form *Citromyces* (Fig. 1 and 2, pl. 203).

The *Citromyces* culture, once obtained and subcultured on sugar agar, vegetates abundantly. It has retained the characteristics of the bacterial cultures of *E. coli;* and, moreover, *coli* exists abundantly on the surface of the agar where it is formed by the segmentation of *Citromyces* mycelial filaments.

Seeded on Endo agar, the *Citromyces* culture turns it pink.

Figure 3 is a photograph of a *Citromyces* preparation made in a region rich in bacterial elements. Among the bacilli, we see germinative masses in the process of forming and others gathered in clusters that will constitute *Citromyces sclerotia.*

To transform the colibacillus cultures into *Penicilium*-type mold, I inoculated the cultures into the guinea pig and cultivated the pulp of its organs on agar. From the third day, *Penicilium* appeared in the cultures (Figs. 4, 5, 6, 7, 8, pl. 203).

This *Penicilium* form is identical to that of human organic mold (Pl. 174, 175) and to that obtained by culturing cancerous tissues (Pl. 196) or by transforming tuberculosis cultures.

The *Citromyces* form is, on the other hand, identical to that of human organic mold (Pl. 176) and to that obtained by culturing tuberculous lung fragments (Figs. 2 to 6, pl. 173).

I isolated the *Citromyces* form in about fifteen cases of tuberculosis; among these cultures, a dozen caused Endo agar to turn pink; they were very virulent for the guinea pig, which succumbed after ten to fifteen days after subcutaneous inoculation and presented a skin ulceration at the injection site.

I have not determined whether the *Citromyces* forms thus obtained derived directly from the tuberculating mold or from the organic mold surrounding the tubercles of the cultured lung fragment.

It follows from these facts that the colibacilli normally found in the mouth and intestine of healthy humans, and especially the colibacilli that exists in the lesions characterizing many human conditions believed to be caused by this bacterial element, *comes from the mycelial segmentation of an abnormal form of human organic mold.* Colibacilli is therefore a witness to the disease and the transformation of the specific organic mold, but not its initial cause; it is an element born spontaneously in the organism and not an infectious germ from the external environment.
These facts imply that each animal species must have its own coli form, linked to the specific organic mold; these different coli species must therefore possess characteris-

tics that differentiate them. Bacteriologists have, in fact, noted the existence of several races of coli that differ from each other in the chemical reactions they carry out in the environments where they are cultivated. I have noted the existence of a horse coli and a dog coli; I have only made this observation occasionally and have not systematically sought to isolate the coli forms of the various animal species; they must be obtained in cultures of the *Citromyces* form of the organic mold of these different species.

It must therefore be concluded that human diseases characterized by lesions in which only one bacterial type of the *colibacilli* form vegetates are, at least in the vast majority of cases, spontaneous autogenous diseases of the specific organic mold. It must nevertheless be admitted that an organism can be infected by the organic mold of another animal species and be invaded by the *colibacilli* form of the organic mold of that species.

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ACTINOMYCOSIS

It is believed to be due to an infection of the animal or human body by the spores of a mold of the *Oospora* type: *Actinomyces bovis (or Nocardia)*. This mold is found in nature on the surface of cereal ears where it vegetates as a saprophyte or parasite.

From the observations I have made, it follows that, at least for the culture of *Nocardia bovis* that I studied, this mold is an abnormal form of organic beef mold; it is a vegetative form of a special type, differing from the type of tuber-forming mold or cancer-causing mold.

The cultures of *Nocardia bovis* used for this research are agar cultures provided to me by Professor Legroux of the Pasteur Institute.

Inoculated in matrass of preserved peptone and glycoseed veal broth, stored at a temperature of 20 to 22°, these cultures easily gave me bacterial development which, after eight to ten days, formed a light and fragile veil of reddish color; this veil, placed on a slide and examined under a microscope, shows its mycelium with long filaments, some branches of which are terminated by balls or oval masses and others by groups of already very distinct sterigmata (Fig. 1, pl. 104). This veil, transferred to sugar agar (maltose, glycose), quickly gave rise to a *Penicilium* form which did not reach complete development (Fig. 2, 3, pl. 104).

It was only by successive subcultures of the latter that I obtained the perfect form which is identical to the *Penicilium* form of the organic moisture of beef.

Beef actinomycosis grains are therefore colonies of an abnormal vegetative form of the organic mold of beef. The mold constituting these grains therefore has close specific

relationships with the tubercular mold since it has the same origin. This explains why it has been observed (Délearde) that filtered *Nocardia* cultures cause fever in tubercular animals and that tuberculin injected into animals carrying actinomycosis causes the same reaction as if these animals were tubercular.

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According to these facts, the agent that vegetates in actinomycosis grains has nothing in common with the mold of the genus *Oospora* that vegetates on the ears of grasses. *Actinomycosis* is a spontaneous disease of the animal and not a disease contracted by contagion.

It follows that the *Nocardia* of cattle must be specifically different from the *Nocardia* of man and that there exists, between these two species, the same difference that exists between the human tubercular mold and the bovine tubercular mold.

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SEPTIC VIBRIO

The septic vibrio is considered the agent of gas gangrene or gangrenous septicemia. It is also observed in gangrene following trauma and even following surgical operations performed with the usual aseptic precautions.

The habitat of the septic vibrio is indicated to be earth, street mud, etc.

My study of the septic vibrio led me to the knowledge of two of its gonidian forms of hyphomycetes, one of which is characteristic.

For my transformation studies, I used cultures of anaerobic septic vibrio provided to me by Professor Legroux of the Pasteur Institute The transformation of the vibrio into a hyphomycete is easily achieved by inoculating, in the presence of air, an anaerobic culture in a matrass of glycosed peptone veal broth kept at room temperature (about 18°). After one to three weeks, the formation of small colonies the size of a pinhead, suspended in the liquid, is observed in the broth, which remains clear. These colonies, transferred to sugar agar or carrot, quickly give the characteristic gonidian form of the septic vibrio photographed in figures 1 and 2 of plate 205. In the matrass of broth, the small colonies grow, become confluent and manage to give rise, on the surface of the liquid, to the aerial fructifications of the hyphomycete; they form on the surface of the liquid a white, mammal-shaped, slightly pink carpet which, later, takes on a darker, rusty pink tint; Drops of clear, reddish-colored liquid form on its surface, identical to the serosity that flows from gangrenous tissue in animals.

A gonidial form identical to that of the septic vibrio can be isolated from cultures of the tetanus bacillus; this form is distinguished from that of the latter by the characteristics

of its culture, which remains creamy white, does not become rusty pink, and on which droplets of red liquid do not form.

This gonidial form of the septic vibrio, which I call *Corymbisporium*, has fruiting bodies in which the spores, with their more or less long pedicle, have the shape of a bell

clapper and are arranged in clusters (Fig. 2, plate 205). The mycelial filaments of the mold are, over a great length, grouped in large parallel bundles (Fig. 1, plate 205).

To obtain the *Penicilium* form, I had to use the process of passing the culture through the guinea pig's organism. As soon as the inoculated animal had succumbed, the pulp of its organs, lung, liver, spleen, was cultured on agar; the *Penicilium* form appeared on the third or fourth day.

At first, I was only able to obtain the rudimentary *Penicilium* form represented in Figs. 3, 4, 5, plate 205. Later, I obtained the perfect form.

In addition, the following are collected from the cultures:

1. A gonidial form *Mucor* with long gonidial stalks

2. A *Sterigmatocystis* form with relatively short, stocky gonidiophores.

3. An *Aspergillus* form with short, stocky gonidiophores.

4. A *Monosterigmata* form.

Identification of the septic vibrio.

The identification of the septic vibrio is made reliably by the six gonidial forms which have just been indicated, and which are those of the organic mold of the carrot.

The septic vibrio is the bacterial culture of the *Corymbisporium* form of carrot. This form is easily obtained (Fig. 18, pl. 260) by culturing uncontaminated carrot pulp on agar; once the mold has developed, its spores are cultured in broth at 18-22° to return it to the bacterial state. We thus see that the culture obtained is a typical culture of septic vibrio. This culture is very virulent, but does not possess the odor of anaerobic cultures of septic vibrio; to reproduce all the characteristics of septic vibrio cultures, it is obviously necessary to accustom the bacterial culture of the *Corymbisporium* form of carrot to the rarefaction of oxygen.

The *Aspergillus* form of carrot also gives a bacterial culture whose elements are almost identical to those of septic vibrio cultures.

The *Penicilium, Monosterigmata, Sterigmatocystis, Aspergillus*, and *Mucor* forms of carrot organic mold are easily obtained by growing the root pulp or organ pulp on agar

from a guinea pig inoculated with carrot juice; examination of these forms shows their identity with those derived from cultures of the septic vibrio.

Since the organic mold of the carrot is also the original source of the rabies and scarlet fever viruses, the same gonidial forms must reproduce by transformation of the virus isolated from the patients. This is demonstrated by the experiment, as will be seen in the study of rabies and scarlet fever. By culturing the brain substance of a dog suffering from street rabies, I isolated the *Corymbisporium* form from the carrot. However, *all the organs of this animal were invaded by the septic vibrio.*

These various results were obtained too late to be supported by photographs. These will be produced in a later publication.

TETANUS

The tetanus bacillus exists in soil, street mud, silt, etc. It is considered anaerobic. It appears either as a bare rod or as a rod ending in a spore (pin-shaped appearance).

As with the septic vibrio, tetanus cultures can easily be transformed into hyphomycetes of various gonidial types. One of these types is characteristic and probably represents the very form in which tetanus vegetates in anaerobic cultures; its fruiting bodies constitute the pin-shaped bacilli (Figs. 1 and 2, pl. 206).

The nature of the tetanus pathogen has been determined by the following studies:

1. Morphological study of the cultures.

The immediate origin and nature of the tetanus bacillus can easily be seen by examining, under high magnification, after staining, the deposit at the bottom of a tube of anaerobic culture aged fifteen days to one month; the result of an observation of this kind has already been described and represented (Fig. 2, pl. 2); we see the pin-shaped bacilli still attached to the mycelium which gives rise to them.

2. Transformation of anaerobic cultures of the tetanus bacillus into various gonidial forms of the tetanus mold.

I obtained various gonidial forms by the following processes:

A. An anaerobic culture of tetanus, which Professor Legroux of the Pasteur Institute had made available to me (*tetanus M*), is inoculated in a flask, away from the air, in half a liter of broth; when the culture placed in an incubator at 38° has produced an abun-

dant deposit, it is distributed, after shaking, into sterile 60 cubic centimeter tubes which are subjected to centrifugation; the clear liquid is decanted and the centrifugation pellet is inoculated on various solid media, sterile dung, cooked and sterile beef, sugar agar, etc., in the presence of air.

In these three environments, the characteristic gonidial form developed, the photograph of which is shown in Figures 1 and 2 of Plate 207.

It is a mold that appears in the form of a thick, tight, milk-white turf at first, then cream-colored, mounded, 2 to 6 millimeters thick; it has a *Corymbisporium* gonidial form identical to that of the septic vibrio (Fig. 2, plate 205), but differs from the latter in that it does not turn a bright rusty pink color and does not emit reddish droplets on its surface **(1)**.

It is likely that this is indeed a characteristic gonidial form of tetanus, because similar club-shaped, pedunculated spores are found in anaerobic cultures of tetanus (Fig. 2, pl. 2).

However, a control is necessary to clearly differentiate it, by gonidial transformations, from the *Corymbisporium* form of the septic vibrio.

B. I obtained the *Penicilium* form by direct transformation of the tetanus toxin by the following process:

An anaerobic culture of tetanus is filtered through a fine porcelain candle placed on an Erlenmeyer flask. Once the filtration is complete, the apparatus is kept intact without uncorking the flask, and stored at room temperature (18°). After about eight days, the external surface of the candle becomes covered with small colonies of *Penicilium;* when subcultured onto agar, this gives an abundant culture; the form of this *Penicilium* is shown in Figures 7, 8, and 9 of Plate 207.

C. The bottom of the tube of the total anaerobic culture used for the previous transformation (B) was spread on sugar agar and the tube stored at room temperature; after about a month, a yellowish-brown colony developed on the agar; it was a form of *Aspergillus,* some of whose fruiting bodies were of the pure *Aspergillus* type and others identical to those shown in Figures 4 and 5 of Plate 208. Subcultured onto another tube of agar, this colony gave rise to a short, egg-yellow-colored turf, which quickly spread over the entire surface of the agar, then liquefied; the culture had the characteristic stinking odor of anaerobic tetanus cultures; in successive subcultures, this odor did not recur.

(1) *I obtained the spontaneous development of an identical mold, presenting this gonidial form characteristic of tetanus, in agar cultures of the tormented tissue of a leproma and in a culture on a carrot of pulp of cancerous breast tissue.*

D. I obtained, indirectly, the *Penicilium* (Fig. 5, 6, pl. 207) and *Sterigmatocystis* (Fig. 3, 4, pl. 207) forms by culturing on agar the pulp of the organs of a guinea pig killed by injection of anaerobic total tetanus culture.

I later obtained much clearer photographs of the *Penicilium* form, but too late to include them in this work. They will appear in another publication.

Inoculated into animals, these various vegetative forms do not confer tetanus on the animals; only the original anaerobic bacterial cultures possess this power, which they lose as soon as their form changes.

3. Study of spontaneous tetanus.

Doctor Valleteau de Mouillac, of Cherbourg, had the opportunity to observe a case of acute spontaneous tetanus, not resulting from an injury, which caused death in thirty-six or forty-eight hours in a twelve-year-old child. He sent me blood taken twelve hours before death. This blood contained an enormous mass of vegetative elements, a photographic representation of which is contained in Figure 1, Plate 208. There are germinative masses giving rise: one, H, to nail-shaped spores K; the other, D, to a mycelial filament B, bearing short-stalked spores F, in the process of development; longer-stalked spores, having the characteristic shape of the tetanus bacillus, are numerous in all parts of the preparation (C). This figure shows that the tetanus bacillus formed in the body is the pedunculated spore of the tetanizing fungus developed on mycelial filaments emanating from germinal masses. Note here that the elements depicted in bacteriology treatises as tetanus bacilli, emitting cilia, are small germinal masses in the process of vegetation and that the cilia are the mycelial filaments, which they always emit. The cilia of the septic vibrio and the typhus bacillus are of the same nature.

The patient's blood, inoculated in glycosylated peptone calf broth, gave rise to a cloudy, egg-yellow culture, on the surface of which developed a veil of mold of the same color consisting of a rudimentary form of *Aspergillus*, including various fruiting bodies are represented in Figures 4, 5, 6, Plate 208.

The mycelium, represented in Figure 3, Plate 208, has a large number of spores pedunculated in the shape of nails and an enormous quantity of zymospores attached to the walls of the mycelial filaments.

A part of this veil, transferred to carrot, gave rise to the same yellowish mold with short hairs, which liquefied in places after two to three weeks. In the liquefied places, a second hairy mold, snow-white, short (1 to 2 millimeters high), developed secondarily.

Examined, it was composed (Fig. 2, Plate 208) of long, creeping, slender filaments, bearing laterally spores pedunculated, identical to tetanus bacilli.

Another part of this veil, transferred to agar, produced the same egg-yellow mold as the *Aspergillus* culture obtained with the anaerobic tetanus bacterial culture used in the previous transformations B and C; like the latter culture, it liquefied after two to three weeks; transferring the creamy yellow matter resulting from this liquefaction back to agar, the *Penicilium* form represented in figures 7, 8, 9, 10, pl. 208, developed on it.

This same creamy yellow matter, inoculated into the guinea pig, proved very virulent; it killed it in about twelve days. The pulp of the organs of the inoculated guinea pigs was cultured on agar.

The pulp of the lung gave rise to a *Penicilium* (Figs. 1, 2, 3, plate 209) identical to that which developed spontaneously by direct transformation of this creamy matter; on the pulp of the liver developed a *Mucor* of the racemosus type represented in figures 4 and 5, plate 209.

In order to verify that there is indeed a link between the *Penicilium* form and the *Mucor* form, the latter was inoculated into eight guinea pigs which succumbed after seven to twenty days; the culture of the pulp of their organs gave rise, without exception, to the *Penicilium* form already obtained twice by direct and indirect transformation of the yellow mold; the *Penicilium* form resulting from *Mucor* is represented in figures 6, 7, plate 209.

We must therefore conclude from these concordant facts that, in this case of spontaneous tetanus, gonidial forms were obtained identical to those obtained by transformation of tetanus cultures.

4. Study of Tetanus Toxin.

This study includes three series of research:

The first concerns the transformation of fresh toxin into hyphomycetes by inoculation into animals and culture of the pulp of their organs. The second concerns the natural evolution of tetanus toxin, its transformation in aerobic culture, and its spontaneous transformation into hyphomycete. The third concerns the indirect transformation of aerobic tetanus cultures into hyphomycetes by passage through the guinea pig's body.

Transformation of fresh tetanus toxin Into hyphomycete by the indirect route.

The nature of tetanus toxin has already been studied on another page (page 188). It has been demonstrated in this study that the toxin is constituted by the zymospores of

the tetanus mold and that, when it is injected into animals, it is not an intoxication that is determined, but tetanus itself, as does the complete culture; the zymospores that the toxin contains are in fact capable of ensuring the reproduction and multiplication of the mold as well as the complete unfiltered culture. The decisive proof of this assertion lies in the fact that by culturing the pulp of the organs of a guinea pig that died after injection of tetanus toxin, most of the gonidian forms of the tetanus mold are obtained and, in particular, one of the most characteristic; that which is represented in figures 1, 2, 3, 4, 5, of plate 206, was precisely obtained by this process. Stalked spores, identical to the pin-shaped tetanus bacilli, form the young spore-bearing arborizations (Figs. 1, 2, 3); these, when fully developed, take on the appearance of a fruiting arborization of *Peronospora* (Figs. 4 and 5) **(1)**.

A culture of this *Peronospora* spontaneously transformed into the *Penicilium* form, taking on a color that was first brown and then green; this *Penicilium* form is identical to those that were obtained by other means, total cultures, spontaneous tetanus, etc.

At the same time as this gonidial form, the culture of the organs animals inoculated with fresh toxin makes it possible to obtain most of the other gonidial forms of tetanus mold: *Penicillium, Aspergillus, Monosterigmata, Mucor,* etc.

Natural evolution of tetanus toxin in aerobic culture. Direct transformation of this into hyphomycetes.

It has also been demonstrated in this study, carried out with freshly filtered tetanus toxin that the Pasteur Institute made available to me, that *the toxin preserved in contact with air develops and vegetates*; the zymospores agglutinate into small germinative masses; at a given moment, these are large enough to develop mycelial and bacillary vegetation. Figure 6 of Plate 206 represents the form of this mycelial vegetation in the toxin from the Pasteur Institute; the bacilli have lost the form of the typical tetanus bacillus; The culture, which has taken on a yellowish tint, still develops the fetid odor of tetanus cultures, even in a subculture; but, inoculated into a guinea pig, it no longer causes clear tetanic phenomena in it; it kills it in ten to twelve days without the animal showing any clear tetanic rigidity, except perhaps near death, the moment when this rigidity ceases to be characteristic.

This aerobic culture of tetanus transferred to agar, spreads rapidly in the form of a creamy, yellow material, which invades the entire surface. This material, scraped with a spatula and inoculated again in two or three small piles, became cove- red with a short, brown mold, which invaded the entire tube.

(1) *Figures 7 to 12 of Plate 206 relate to other diseases (chickenpox and typhoid fever).*

Examined in various regions, this mold consisted of:

In some places, by large segmented mycelial filaments, bearing a few large chlamydospores, similar to the first large mycelial filaments emitted by brown rot of the potato (Fig. 1 to 5, pl. 147).

In others, by a similar mold or with smaller mycelial filaments, emitting thin branches bearing long spores, often a little arched, but small, of the *Fusarium* type, and similar to those often borne by the first mycelium of brown rot of the potato (Fig. 2, pl. 147).

Finally, in some places, the mold was in the process of passing to the *Penicilium* form and was already emitting gonidiophores bearing two to three sterigmata.

The mold developed on the creamy material from the aerobic tetanus culture therefore exhibits characteristics of an early mold that develops on brown rot (*Phytophtora infestans*) of the potato. These molds, cultivated to obtain more perfect development, are currently under study.

Indirect transformation of aerobic tetanus cultures into hyphomycetes.

Thus, one can easily transform an anaerobic tetanus culture into an aerobic culture; but the latter has lost its tetanizing power. Using this culture, I was able to obtain different gonidial forms that allowed me to identify the tetanus mold, that is, to rediscover its original form.

An aerobic culture was inoculated into a batch of guinea pigs and the pulp of their organs was cultured after death. In addition to the gonidial forms already obtained previously, three new forms developed on these cultures:

1st, a very particular form, identical to that represented in Figure 5, Plate 146, and which I classify in the *Verticilium* type;

2nd, *Verticilium alboatrum or solani*;

3rd, a *Mucor* form, identical to that represented in Figure 5, Plate 149;

4th, a *Monosterigmata* form identical to that which I obtained in the study of smallpox and foot-and-mouth disease (Figs. 13, 14, 15, pl. 271, and Fig. 1, pl. 275).

I thus had at my disposal, to begin the study of identifying the tetanus mold, eight different gonidial forms:

1. A form of the *Corymbisporium* type.
2. A form of the *Peronospora* type.
3. A *Monosterigmata* form.

4. Two forms of the *Verticilium* type.
5. A *Mucor* form.
6. An *Aspergillus* form.
7. A *Penicilium* form.

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Identification of the Tetanus Mold.

This identification was not achieved all at once. Here are the elements that gradually contributed to it:

I had long observed that the *Penicilium* form of the potato, reduced to the bacterial form by broth culture, gives rise to numerous pin-shaped bacilli, analogous to the tetanus bacillus.

As soon as I had obtained the *Penicilium* form of the tetanus mold, I sought to identify it with one of the many species I possessed in this gonidial form. I noted from that moment that it was the *Penicilium* form of the potato that achieved the identity.

The identification became almost certain when I obtained the Verticilium form of tetanus mold identical to the Verticilium form of the potato shown in Figure 5, Plate 146, and a second, very characteristic form, *Verticilium alboatrum* or *V. solani.* At this point, having already isolated the *Monosterigmate* and *Mucor* forms of tetanus mold, I compared them and found them to be identical to those of the potato, which I also possessed. I also recognized a complete identity between all these gonidial forms of tetanus mold and those of cowpox, smallpox, and foot-and-mouth disease, diseases caused by the organic mold of the potato.

It remained to establish the identity between the *Peronospora* form of tetanus and that of the potato The gonidia of the tetanus *Peronospora* form closely resemble the oval gonidia of *Phytophtora infestans*, but differ from them in their pedicle. I succeeded in obtaining the perfect potato *Peronospora* form by culturing the organs of a guinea pig inoculated with tuber pulp. I noted that this form, identical to that of the *Peronospora* obtained by direct culture of *Trichophyton crateriforme* on egg yolk (Fig. 1, pl. 301 bis) and which is confused with *Botrytis cinerea,* had such common points with the tetanus *Peronospora* that there was reason to consider them as two different aspects of the same *Peronospora*. The transition from tetanus *Peronospora* to the perfect form is under study.

But there was another excellent way to check the accuracy of identification; it is the comparison of bacterial forms. This control had to include two operations.

1. The transformation of anaerobic tetanus cultures into different bacterial forms of the tetanus pin bacillus, and the comparison of these forms with those of the organic mold of the potato; to this end, the transformation is carried out by an identical process for both molds; two guinea pigs are inoculated, one with a tetanus culture or with its toxin, and the other with an emulsion of potato pulp carefully taken from the center of a tuber **(1)**. The pulp of the animals' organs is cultured on agar.

A bacterial culture of the inoculated pathogen always develops in several tubes, extending downward from the inoculated region to the bottom of the tubes. The bacterial forms of these cultures are compared, and they are inoculated into broth to also compare the bacterial forms that appear there. The same forms are thus found in both cases. The identification therefore continues down to the bacterial forms.

2. The transformation of bacterial cultures of the organic mold of the potato into anaerobic tetanus cultures. This study is currently underway with a view to obtaining anaerobic cultures possessing the tetanus pin bacillus and the tetanizing power, either directly using tuber pulp, or by transformation, using the Kitazato process, of aerobic bacterial cultures already obtained from this pulp.

There is certainty of obtaining this culture because it develops naturally in tubers: it is the culture that determines butyric fermentation in rotting tubers.

Examining the rot- ting juice of a tuber exhibiting this fermentation, we find numerous tetanus bacilli and bell-shaped bacilli like certain forms of the septic vibrio. This butyric fermentation of the potato has been attributed to *Bacillus amylobacter* or *Bacillus solaniperda*. In reality, it is caused by a bacterial form of organic potato mold that takes on this fermentative character because it develops in a region of the tuber that is significantly deprived of oxygen.

In summary, the identity of the gonidial forms obtained by transformation of tetanus cultures and gonidial forms of potato mold, the identity of the bacterial forms of these two molds constitutes a body of evidence that allows us to conclude with certainty that tetanus is caused by a bacterial form of potato mold.

(1) *Or with the spores of the Aspergillus form of the potato.*

Consequences of the identification of tetanus mold.

To achieve the identification of tetanus mold, I had to accumulate multiple pieces of evidence, because I refused to admit that potato mold, which had already been determined to be the original source of the pathogens of vaccine, smallpox, and foot-and-mouth disease, could at the same time be that of the pathogen of tetanus.

This identification, which can now be beyond doubt, gives us the explanation for the following unexplained phenomena:

1. It has been found that hypodermic injection of aseptic liquids can cause tetanus. It has been concluded that tetanus spores exist, in a latent state, in the human body. These spores do indeed exist. Indeed, tetanus is a bacterial form of the organic mold of the potato; any individual who has undergone smallpox vaccination, or who has contracted smallpox, is affected by a long-term mycosis persisting as long as the vaccination state and consisting of the vegetation of the organic mold of the potato. An aseptic hypodermic injection can cause tetanus if it disorganizes and suspends, in the injected liquid, spore-bearing elements of the vaccine mold capable of giving rise, by transformation, to a tetanic culture.

2. The same explanation applies to traumatic tetanus and spontaneous tetanus; the latter can be explained by a vegetative growth of the vaccine mold; however, it is also possible that it can be caused by infection with potatoes and even by ingestion of cooked potatoes, fermented or not after cooking.

Based on these facts, it is not certain that tetanus following a deep, punctured wound (war wounds and others) comes from an external infection; it can come from elements of the vaccine mold detached from their connections and passing into a state of active bacterial evolution.

As a natural consequence of these facts, a study of the following points is currently being carried out:

1. Does smallpox vaccine confer protection against tetanus? The fact of the spontaneous development of tetanus by injection of an aseptic liquid indicates that this protection must not exist and that it appears rather that it is the presence of the vaccine mold that confers the subject the ability to contract tetanus. These facts are currently under study.

2. Does an injection of antitetanus serum immunize against the vaccine mold?

LEPROSY

The pathogenic agent of leprosy is Hansen's bacillus. In reality, this bacillus is only one of the elements of the pathogenic agent, which is a mold whose vegetation in the body constitutes, at certain points, nodules called lepromas.

Due to the difficulty of obtaining lepromas, I have not been able, until now, to conduct a complete study leading to the identification of the mold that causes leprosy.

Figure 1, Plate 210, shows the vegetation of the pathogenic mold in a leproma of the forearm the size of a small hazelnut, taken by biopsy from a black woman from Martinique. The mycelium bears fruiting bodies in very visible pedunculated balls. The leproma being isolated from the surrounding connective tissue, then torn and ground, its pulp is cultivated on carrot and on agar. On all the culture tubes I obtained the same mold, blue-green in color, of the *Penicilium* form. The mycelium which vegetates on the surface of the carrot emits fine, slender filaments, the segmentation of which forms the *Hansen bacilli* which are observed in their intricacy (at the top of figure 2); the largest mycelial filaments bear fructifications in the form of pedunculated balls similar to those which are observed in the leproma itself; the presence of these same fructifications in the balls in the mold which develops in the *Penicilium* form indicates that, probably, the mold which constitutes the leproma vegetates in the rudimentary *Penicilium* form, the groups of sterigmata remaining in the form of balls and not reaching their complete development.

The fully developed *Penicilium* form is shown in Figures 3 and 4, Plate 210. I have not yet made a sufficient study of this *Penicilium* to identify it and determine its origin.

GLANDERS

I studied glanders using two cultures called glanders L and glanders V, which were provided to me by Professor Legroux of the Pasteur Institute.

The glanders L culture, subcultured on various solid media, spontaneously transformed, on egg yolk, into the *Penicilium* form shown in Figure 1, Plate 211.

The glanders V culture treated in the same way gave rise, on carrots only, to the *Aspergillus* form shown in Figure 2, Plate 211. To obtain the *Penicilium* form, I had to resort to the process of indirect transformation by passage through the guinea pig's organism. The pulp of the organs (lung, liver, spleen) of a guinea pig inoculated with the glanders V culture being cultivated on agar, the *Penicilium* form developed on all the tubes. Figu-

res 3 to 7 represent this *Penicilium* form developed on the pulp of the spleen; this form is identical to that already obtained (Fig. 1) by transformation of the glanders L culture. These gonidial forms have not yet been identified.

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DIPHTHERIA

The diphtheria bacillus is only one of the elements (mycelial segmentation element) of the mold that constitutes the actual pathogenic agent of the disease. This mold can be easily isolated in several gonidial forms; the study of these leads to knowledge of the original source of the diphtheria virus; the pathogenic mold is revealed to be, by various characteristics, the organic mold of barley.

The first diphtheria culture whose development I studied was provided to me by Professor Bezançon; it came from the Pasteur Institute and was called the Weiss-Martin culture. This culture, made by puncturing on agar, was one month old and presented on its surface a very light whitish down consisting of the mold of the *Citromyces* type represented in Figures 2, 3, and 5, Plate 212; transplanted on agar, it developed in the same gonidial form (Fig. 1, 4, plate 212). The mycelium of this mold was shown to be formed of elements identical to diphtheria bacilli provided with polar granulations and placed end to end (Fig. 2, plate 212), the mycelial filaments segmenting by scission in the middle of the spaces strongly colored in blue (persistence of basophilic protoplasm at this level; black coloration in the figure); each detached element has, at its two ends, a small mass of basophilic protoplasm which forms a ball and constitutes a polar granulation.

This mycelial origin of the bacilli is further demonstrated in the study that was made of the development of diphtheria toxin and its return to normal bacterial culture. During this development, the formation of bacilli by mycelial segmentation was exposed. Figure 7, plate 116, shows a mycelial filament formed of bacillary elements ready to disintegrate in this filament; each segment which, once free, will be a bacillus, already has its two polar globules formed.

The diphtheria false membrane is constituted by the pathogenic mold developed either in the *Citromyces* form or in the rudimentary *Penicilium* form. By examining under a microscope fragments of false membrane dissociated on a slide and stained with dahlia, one sees numerous mycelial filaments, germinative masses and large, fairly rare spherical balls in which the four or five already formed Citromyces sterigmata are very clearly distinguished.

Subsequently, I obtained the diphtheria mold in several gonidial forms by the following methods:

1. By direct culture of crushed false diphtheria membranes spread on sugar agar. I thus cultivated about ten false membranes which all gave rise to the *Citromyces* form or the *Penicilium* form; thus, one of them gave rise to the *Citromyces* form represented in figs. 6, 7, 8, of plate 212; a second gave rise to the *Penicilium* form and the *Monosterigmata* form (Fig. 1, 2, pl. 213); a third gave rise to the *Penicilium* form of figures 3, 4, 5, plate 213; a fourth gave the *Penicilium* form represented in figures 1, 2, 4, 6, 7, 8, 10 of plate 214; figures 3, 5, 9, 10, 11 of the same plate 214, represent the *Penicilium* form obtained by direct transformation of a diphtheria culture from the Pasteur Institute.

2. By direct transformation of diphtheria bacillus cultures. A culture of diphtheria bacillus (preferably on agar) is spread on various moist solid media, egg yolk, meat, casein, etc.; after a variable time, which can exceed a month, it always manages to transform, on one of the media, into aerial mold. Figures 3, 5, 9, 10, 11, plate 214, represent the *Penicilium* form, developed on cooked beef fragments, of a diphtheria culture on agar made available to me by the Pasteur Institute. This form is identical to those obtained by culture of false membranes (Fig. 1, 2, 4, 6, 7, 8, 10, plate 214).

3. By inoculating the guinea pig with diphtheria cultures and culturing the animal's organ pulp on agar after death. This produces, on the organ pulp, from the third or 4th day, a *Penicilium* form identical to those in plates 213, 214, and 215.

4. By inoculating the guinea pig with diphtheria toxin and culturing the organ pulp on agar. I mention this process here because, in addition to the *Penicilium* form of the diphtheria mold that it allows us to obtain, it demonstrates that inoculating animals with diphtheria toxin does not produce poisoning by a soluble product secreted by the bacillus, but causes diphtheria itself and the development of the diphtheria mold, just as a complete, total culture of the bacillus does.

By culturing the pulp of the organs of the guinea pig that died after injection of diphtheria toxin, I obtained:

1. The most complete type of the *Penicilium* form of the diphtheria mold (Figs. 1 to 10, pl. 215).

2. Another mold of light brown ochre color, presenting a gonidial type which, on certain filaments, presents the *Monosterigma* type, and, on others, forms which seem to evolve into the *Acaulium* or *Penicilium brevicaule* type (Figs. 11, 12, pl. 215). This mold will be discussed later.

Here are obtained and known some of the different gonidial forms that the diphtheria mold can take: *Citromyces, Penicilium, Monosterigma* forms, the latter in two different types or aspects.

Identification of the diphtheria mold or search for the source of the diphtheria virus.

This identification research had to be pursued in parallel with that of three other pathogenic molds, paratyphus (Para A and B) and typhus, because the *Penicilium* forms of these three molds appeared to correspond to those of the *Penicilium* form of the organic mold of the cereal food plant group; the research was first located between the organic molds of wheat, barley, rye, and oats.

The gonidial and bacterial forms of the organic mold of oats allowed it to be identified fairly easily with typhus.

The identification of diphtheria and paratyphoid molds was therefore reduced to a comparison with the organic molds of wheat, barley, and rye.

The identification research consists of:

1. In the comparison of the gonidial forms of diphtheria and paratyphoid A and B molds with those of organic mold of wheat, barley, and rye.

2. In the comparison of the morphological characters of bacterial cultures of wheat, barley, and rye with cultures of diphtheria bacilli and paratyphoid B.

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3. In a study of the action of bacterial cultures of wheat, barley, and rye on inoculated animals.

1. Comparison of the gonidial forms of diphtheria and paratyphoid molds with those of the organic mold of wheat, barley, and rye.

I had already obtained various gonidial forms of the organic mold of wheat, barley, and rye, during the study of the diseases that affect these cereals. These gonidial forms were obtained: for wheat, by culture on agar, either of spores of bunted wheat grains or of young aseptic wheat shoots reduced to pulp**(1)**, or of the pulp of the organs of a guinea pig inoculated with spores of bunted grains or with the pulp of young leaves; for rye, by culture on agar of rye grains reduced to a moist pulp or of the pulp of the organs of a guinea pig inoculated with this pulp; for barley, by culture on agar of spores of

(1) *To obtain them, grains of wheat or other cereals are sown under a sterile bell jar in sterile moist sand, and the culture is kept humid at 22-24°.*

Ustilago hordei or of pulps of young aseptic barley leaves. By these methods, different gonidian forms are easily obtained; those of wheat and barley have already been described in connection with the diseases of these plants.

Plates 216 and 217 contain various types of the *Penicillium* form of wheat mold, Plate 218 contains those of barley mold, and Plate 219 contains those of rye mold. For this identification study, it is necessary to compare each of the figures in Plates 214 and 215 (*Penicillium* form of diphtheria mold) with the figures in Plates 216, 217, 218, and 219. In addition, the figures in these last plates must be compared with those in Plate 222 (*Penicillium* form obtained by transformation of bacterial cultures of Paratyphoid B).

From this comparison, it results:

1. That the *Penicilium* forms of the organic mold of barley and wheat, very similar and delicately differentiated, present a striking resemblance to the *Penicilium* form of the diphtheria mold. This resemblance continues even in certain elementary forms presenting a particular appearance (Fig. 9, 10, pl. 216, wheat; fig. 6 and 7, pl. 161, barley; fig. 3, 4, 5, pl. 213, fig. 1, pl. 214, diphtheria).

2. That the *Penicilium* form of the organic mold of rye, although presenting certain types analogous to those of the *Penicilium* form of the diphtheria mold (Fig. 15, 16, pl.219), is much closer to that of the paratyphoid mold B. Compare for example in this respect figures 1, 2, 3, 4, 5, plate 219 (rye) with figures 1, 2, 3, 4, 5, plate 222 (paratyphoid B); figures 16 and 17, plate 219 (rye) with figure 6, plate 222 (diphtheria).

The search for identification of diphtheria mold is therefore localized to wheat and barley. Certain rudimentary types of *Penicilium* of barley (Fig. 7, pl. 161), which I found identical in a culture obtained by transformation of a diphtheria bacterial culture, indicated to me that identification should be directed towards barley.

The culture of the organs of a guinea pig inoculated with diphtheria toxin (equivalent to inoculation of the complete culture), gave rise to an ochre-colored mold, of *Monosterigmata gonidial* form (evolving into a more complicated form) (Fig. 11, 12, pl. 215), perfectly identical to a mold of the same ochre color and the same gonidial form belonging to the organic mold of barley (Fig. 3, 4, pl. 161).

The identification of diphtheria mold with barley organic mold is therefore established by the latter gonidial form; this identification is confirmed by comparing the bacterial forms and the respective pathogenic action of wheat and barley crops.

2. Comparison of the bacterial forms of diphtheria mold with the bacterial forms of organic mold of wheat, barley, and rye.

Bacterial cultures of the organic mold of various cereal species are easily obtained by inoculating peptone and glycose broth with a little pulp from young aseptic shoots, or with the opalescent liquid obtained by grinding in water a particle taken aseptically from the inside of a grain; experience shows that by quickly flaming a grain of cereal with a flame and immediately plunging it into a little cold water where it is ground, the inoculation of the liquid obtained also gives a pure bacterial culture.

Plate 220 contains, in figures 7, 8, 9, 10, 11, photographs of different aspects of a diphtheria culture reconstituted by spontaneous development, at room temperature, of diphtheria toxin from the Pasteur Institute. In these various forms we note that the culture can contain, in addition to the usual long and short bacilli, a larger bacillary form, two elements of which are visible towards the center of figure 9. Let us note, in passing, an important fact: by examining with a magnifying glass the bacterial clusters of figures 7 and 9, plate 220, and those of figure 5, plate 221 (barley), we can quite clearly distinguish between the bacillary elements the filaments of hyaline substance (agglutinin or bacterial fibrin) which agglutinate them, as the fibrin of blood does for red blood cells.

In the same plate 220, figures 1 to 6 contain photographs of various bacterial forms of organic wheat mold. It can be seen from these figures that these forms have some characteristics in common with those of diphtheria bacilli. There are long and short forms, and the broad bacillary form can be seen (Fig. 2).

In Plate 221, Figures 5 to 10 contain photographs of the bacterial forms of organic barley mold. These forms are morphologically and perfectly similar to those of diphtheria bacilli; there are long forms and short forms: the bacilli are provided with two polar globules like the diphtheria bacillus. It is also possible to obtain the bacterial culture of barley by culturing the diastase of germinated barley in broth. Figures 11 and 12 are photographs of four-day cultures thus obtained; in addition to the bacterial forms of the previous cultures, we can see the large bacillary form already observed in diphtheria cultures (Fig. 9, Plate 220).

Bacterial cultures of organic rye mold (Figs. 1, 2, 3, 4, pl. 221) are distinguished by the presence, at least in broth cultures ten to twenty days old, of coccobacilli with bipolar globules (Fig. 4); in the first few days, the cultures contain long mycelial filaments in the process of bacillary segmentation. Around the third or fourth day, oval masses (Fig. 3) similar to yeast cells are observed, which appear to be formed by agglomeration and can multiply by budding; they are formed, at the beginning (when they are formed by

agglomeration), by a hyaloplasmic mass in which a basophilic nucleus is formed by the addition of basophilic protoplasm from the bacillary elements, which blend into the mass. When bacterial cultures of rye are created by seeding pulp from plant or grain tissue, a fully formed culture can be immediately obtained from these masses.

These different characteristics, notably the presence of specially shaped bipolar cocco-bacilli, clearly differentiate cultures of organic rye mold from cultures of the diphtheria bacillus.

This elimination of rye, in the current comparison and the perfect identity of the bacterial forms of the barley culture with those of the diphtheria culture, therefore lead us to say that the diphtheria mold is a vegetative form of the organic barley mold.

3. Pathogenic action of bacterial cultures of wheat and barley.

The two cultures of wheat and barley being inoculated respectively into two guinea pigs at the same dose of one-half to one and a half cubic centimeters, an immediate difference appears. The bacterial culture of barley is much more virulent than that of wheat; that of barley kills the guinea pig in one to three days, that of wheat in six to twelve days.

But it is above all the characteristic lesions caused by the barley culture that definitively identify it with the diphtheria culture; one notes congestion of the intestinal viscera, congestion of varying intensity of the adrenal capsules; they generally present hemorrhagic foci; sometimes, the congestion is very violent; one notes, in addition, congestive pulmonary lesions and, often, a slight pleural and peritoneal effusion.

The identification of diphtheria mold with barley organic mold, thus achieved by comparing gonidial forms, bacterial forms, and the pathogenic action of cultures, therefore becomes certain: barley is the original source of diphtheria.

Bacterial cultures of barley organic mold therefore exist on the soil and in the manure of stables in which barley straw serves as bedding for the animals, because barley straw, although dry, retains the reproductive elements of the plant organic mold alive. Milk, being almost always contaminated by stable manure, is therefore frequently contaminated by manure containing the culture of barley organic mold, that is, the diphtheria culture.

Barley flour contains, alive, the reproductive elements of organic mold, that is to say elements necessary for the birth of a diphtheria culture. Simply pound a grain of

flambéed barley in a mortar and emulsify a little of the flour obtained in broth kept at 20-24°, to obtain the bacterial culture.

It is therefore necessary first of all, to eliminate diphtheria or reduce the number of cases, to eliminate barley flour from children's diets and to eliminate the use of barley straw as bedding in dairy cow stables.

Note also that the diastase of germinated barley contains the reproductive elements of a diphtheria culture and develops it in the bacillary form in one to three days when it is included in a suitable nutrient medium.

Brewer's yeast, at least the one I studied, is also a vegetative form of the organic mold of barley.

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PARATYPHUS B

The paratyphus B cultures that I studied were made available to me by the Pasteur Institute (Professor Legroux).

I transformed the paratyphus B cultures indirectly, that is, by inoculating the guinea pig and culturing the pulp of its organs. I thus obtained only the *Penicilium* form represented in Plate 222. But this Penicilium form is so closely related to that of the organic mold of rye (Plate 219) that I did not hesitate to conclude that rye is the original source of paratyphus B.

A careful comparison of the figures in Plates 222 (paratyphus B) and 219 (rye) will show the striking resemblance of the two *Penicilium* forms.

The bacterial forms of the first cultures obtained directly by seeding a crushed rye grain may appear different, at first glance, from the paratyphoid bacterial forms. This difference disappears in a rye bacterial culture obtained by passage through the guinea pig's body. A guinea pig is inoculated either with the culture obtained directly by seeding a crushed rye grain, or with the crushed grain itself emulsified in water. After the animal's death, the pulp of its organs is cultured on agar. Around the seeded pulp, a semi-liquid material develops, which is the rye bacterial culture. Examined in this form or in the broth culture where it is transferred, the rye bacterial culture exhibits the bacillary forms of paratyphoid B. These forms, obtained too late to appear in the plates of this work, will be presented elsewhere.

I attempted to identify rye cultures by agglutination tests using *Para B* agglutinating serum.

I obtained a positive result, but not sufficiently conclusive; the rye cultures are indeed agglutinated, but with a low dilution of the serum (approximately 1/50 to 1/80). The results are not significant, but the tests have not yet been numerous enough or varied enough to lead to a definitive conclusion.

We must therefore only remember that the identity of the *Penicilium* forms and the *bacterial forms* indicates that the *Paratyphi B* mold is a vegetative form of the organic mold of rye.

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PARATYPHUS A

I studied *Paratyphus A* on different cultures made available to me by Professor Philibert.

The transformation of bacterial cultures is carried out with the greatest ease by inoculating them into the guinea pig and culturing the pulp of the organs on agar after death. These cultures become covered after three or four days with the *Penicilium* form of the pathogenic mold. This is fairly easy to identify; it is the *Penicilium* form of the organic wheat mold.

Cultures of the pulp of the organs of the inoculated guinea pigs also give rise to a whitish coating that flows to the bottom of the tubes and is a bacterial culture of the paratyphoid bacillus A. This culture, like the original cultures used for the inoculations, identifies perfectly with the bacterial cultures of organic wheat mold. The bacillary forms of these vary in length and do not always appear as short, stocky bacilli, but they often take on this characteristic form, which is observed, moreover, at a certain period in the development of the cultures. Paratyphoid bacillus A also does not always appear as short, stocky bacilli; it can also appear as longer bacilli.

From this double identification, it follows that the original source of the pathogenic agent of *Paratyphoid A* is organic wheat mold.

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TYPHOID FEVER

The pathogen of typhoid fever is a mold that gives rise to *Eberth's bacillus* by segmentation of its mycelium.

The transformation of cultures of the typhus bacillus into aerial mold is one of the first that I was able to carry out; I obtained it by placing a drop of culture on a fragment of damp refractory earth placed in a sterile dish containing a little broth (1 or 2 millimeters high); the fragment became covered with an aerial mold having the form of a

rudimentary *Penicilium* or a *Citromyces* (Fig. 5, 6, pl. 223). This mold, being transplanted several times in succession on carrots, taking care to inoculate only the spores, developed in the clear *Citromyces* form (Fig. 4, pl. 223); I noted that the surface of the carrot was covered with typhus bacilli (Fig. 1, pl. 223) scattered between the mycelial filaments of the *Citromyces*; they came from the segmentation of these.

The culture of *Citromyces* was fragile and had a tendency to melt, that is, to return to the bacterial state; transferred to agar, it underwent this transformation and developed in the form of a creamy material which, when examined, was made up of spherical masses (Fig. 3, pl. 223) gathered in groups of various shapes, placed end to end or isolated, which appeared to be formed by agglomeration of granulations. Some of these masses emitted long filaments (A, B, C, D, E, fig. 2, pl. 223) which were those described and known under the name of cilia of the typhus bacillus; these are, not cilia, but mycelial filaments; whether they are emitted by a spherical mass or by the bacilli themselves, the nature of these filaments is demonstrated by the small spherical fructifications which they bear; They in fact give rise, at many points (examine the figure with a magnifying glass), to very small, short-pedunculated spores which are zymospores and which, once detached, will constitute the fermentative agent, or toxin, of the culture.

Figure 1, showing bacillary elements formed in the culture of the original *Citromyces,* also shows a small spherical mass emitting fine mycelial filaments and isolated spores which each emit a fine filament bearing zymospores. This is one of the usual processes which give rise to zymospores; it has been described elsewhere (*Development of Ferments*).

The existence of these mycelial filaments in bacillary cultures, their shape, and their role in the formation of zymospores will serve us in the search for the source of the typhus virus.

Later, I obtained with much more ease the perfect *Penicilium* form of the typhus mold; it grows abundantly, from the third to the fourth day, on the pulp of the organs of a guinea pig (lung, liver, spleen), which died after inoculation with a culture of Eberth's bacillus.

Plates 224 and 225 contain photographs of various types of the *Penicilium* form from two bacterial cultures of different origins, made available to me by Professor Philibert and bearing the designations, one Eberth **v**erified (Plate 224), the other Eberth-Lardenois (Plate 225). A third culture designated Eberth-Philibert gave rise only to elementary fruiting forms perfectly identical to those in Figures 5 and 6, Plate 223. This is a very distinct and characterized form of *Penicilium*.

Identification of the typhus mold and search for the original source of the typhoid fever virus.

During my research on plant diseases, I inoculated a guinea pig with corn smut spores, with a view to investigating whether the gonidial forms obtained by culturing the pulp of the organs would be identical to those of corn mold. At the animal's autopsy, I was interested in the lesions caused by this pathogenic agent; there were multiple tubercles of the liver, spleen, and peritoneum, and the mesenteric lymph nodes, greatly enlarged, were caseous.

The large intestine also bore quite numerous tubercles embedded in the intestinal wall: *some, opened inside the digestive tract, had caused an ulceration identical to that of typhoid fever. The results of this autopsy were confirmed by new inoculations.*

This fact made me think that typhoid mold could be a vegetative form of corn mold.

I was struck, when I came into possession of the perfect *Penicilium* form of this plant, by its resemblance to the perfect form of the typhic *Penicilium*.

Figure 1, plate 226 (*Penicilium* form of the organic fissure of maize), is a photograph of the elementary form of the termination of one of the branches of the gonidial stalk; it has a special, distinct type, which is found in most gonidial branches. This characteristic device is found in the gonidial branches of the typhus mold and this in most of the figures of plates 224 and 225; it is very clear in figures 1, 3, 8, 10, 12 of plate 224 and in figure 1 of plate 225. The groups of terminal sterigmata have, on the other hand, a particular form (Fig. 3, pl. 226, maize) which is found identically in the typhus mold (Fig. 1, 4, 12, pl. 224; fig. 1, 6, 9, pl. 225).

Finally, the comparison of the general form and other characteristics of the fruiting end shows such a resemblance between the two molds that we must conclude their identity; this continues even in the slender forms of the two molds (Fig. 8, 14, 15, 16, pl. 226. corn; fig. 12, 15, pl. 224; fig. 10, pl. 225, Eberth).

This study has been limited to the comparison of *Penicilium* forms because I have so far only obtained this gonidial form (with the *Citromyces* form) of the typhus mold. But I have obtained other gonidial forms of the organic mold of corn: the *Fusarium* form, which is characteristic (Fig. 14, 15, pl. 159), and the *Mucor* form, which affects the *Corymbifer* type (Fig. 12, 13, pl. 159).

Research is being carried out to verify whether the typhus mold possesses these same gonidial forms.

The identity just explained, following the observation of the nature of the lesions caused by corn smut in the guinea pig, led me to conclude that typhus mold is a gonidial form of the organic mold of corn, even before knowing the bacterial forms of the latter, the research for which was only carried out with a view to verification and to providing new evidence; the result of this research was a confirmation of identity. The bacterial culture of corn is easily obtained by the following processes:

1. By grinding a grain of flamed corn in sterile water and placing one or two drops of the liquid obtained in peptone or glycoseed veal broth, or on agar.

2. By inoculating the sterile pulp of the subcortical tissue or the medullary tissue of the fresh corn stalk in broth or on agar.

3. By inoculating a guinea pig, either with the pulp of stem tissue or fresh corn leaves, or with the liquid obtained by grinding aseptic corn grain, or with corn smut spores;

after death, the pulp of the organs (lung, liver, spleen) is cultured on agar; in addition to the gonidial forms of organic corn mold, a bacterial culture of this mold develops in the form of a creamy material that flows to the bottom of the tubes.

4. By broth culture of the caseous material from the hypertrophied mesenteric lymph nodes of a guinea pig inoculated with corn smut spores.

These cultures are characteristic; they contain a single bacillary form morphologically identical to that of the typhus bacillus. The photograph of this form, taken on two different cultures, is contained in Figures 11 and 12 of Plate 206 (Compare to the bacillary form in Figure 1, Plate 223: *Typhoid bacillus)*.

Having reached this conclusion of identity, I performed subcutaneous inoculations on guinea pigs with the organic mold of corn itself, leaf pulp or an emulsion of pounded grains; the animals sweat ten or fifteen days after inoculation, but no longer present the same lesions as those which received corn smut spores. Rather, congestive lesions with a few tubercles of the liver and spleen are observed, but without tubercles of the intestinal mucosa.

The first culture, a photograph of which is shown in Figure 11, was obtained by inoculating in broth the caseous matter of the hypertrophied mesenteric lymph nodes of a guinea pig inoculated under the skin of a paw with corn smut spores; the second culture (Figure 12) was obtained directly by inoculating the pulp of young corn leaves developed in aseptic culture.

The cultures obtained are morphologically identical to those of the typhus bacillus; comparison of the bacillary forms in Figure 11, Plate 206, with those in Figure 1, Plate

223, shows perfect identity; on the other hand, careful examination of the preparations and bacillary elements in Figure 11 shows:

1. That certain bacilli bear cilia, that is, emit fine mycelial filaments giving rise to short-pedunculated zymospores.

2. That isolated spores give rise to short-pedunculated zymospores.

3. That there are small rounded masses, formed by the union of spores emitting long, sinuous mycelial filaments, giving rise to short-stalked zymospores.

These mycelial filaments, or cilia, are identical to those described in cultures of the typhus bacillus and which are represented in Figures 1, 2, and 3 of Plate 223.

The identity of form of the microbial elements in bacterial cultures of corn or Eberth's bacillus is therefore completed and corroborated by the presence, in both cases, of cilia or mycelial filaments giving rise to the zymospores.

These facts being known, I carried out a new identity check by agglutination tests of the bacterial cultures of corn. So far, these tests have given a negative result; the cultures are not agglutinated.

I inoculated a rabbit with 0.1 cc of corn culture broth (which killed this animal in forty-eight hours at a dose of 0.3 cc to 0.5 cc) and tested the agglutinating power of its serum for typhus bacillus cultures; the result was negative.

From this failure, it should not be concluded that typhus mold has no relationship with organic corn mold; these negative agglutination tests, which I have reported to provide a complete and accurate account of the study carried out so far, are not numerous and varied enough to lead to a definitive judgment; they are continued and multiplied.

In summary:

1. Subcutaneous inoculation of corn smut spores in the guinea pig causes generalized tuberculosis with caseous tubercles and ulcerations of the intestinal mucosa.

2. The Penicilium forms of corn mold and typhus mold are identical.

3. The bacillary forms of corn mold and typhus mold are morphologically identical.

From these facts, I must conclude, at present, that typhus mold is a vegetative form of corn mold; Eberth's bacillus is therefore a bacterial form of corn mold.

The sources of the typhus virus would therefore be:

1. The bacterial elements normally developed in the soil by the roots in corn crops; we have seen that every plant carries out the transformation of nutrients in the soil by the

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bacterial elements formed by its organic mold and originating from the mycelial filaments called root hairs of the rootlets. These bacterial elements are carried by rainwater.

2. The various parts of corn plants thrown onto manure where they rot.

3. The seeds given as food to animals and rotting on the ground.

4. Corn flour diluted in water and remaining there.

5. The spores and zymospores of corn smut spread on the ground and carried by rainwater.

From the preceding statement, it follows that corn smut spores are more dangerous than the normal elements of the plant, and that the handling of subjects infected with smut must be done with caution.

Research is continuing to verify the accuracy of this origin of the typhus virus.

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EXANTHEMATIC TYPHUS

I was able to study exanthematic typhus thanks to the kindness of Dr. Bialomskja, who kindly sent me two inoculated guinea pigs, one with lice carrying the virus, the other with blood from a patient suffering from typhus. I was able to transmit the virus to a long series of animals by inoculating them with a little crushed brain substance from either animals sacrificed at the moment the temperature curve drops (after three weeks to a month), or from animals that died spontaneously.

I isolated the pathogenic agent of typhus from the very animals that Dr. Bialomskja had sent me. These two animals were sacrificed about the twenty-fifth day after their inoculation; a series of other guinea pigs was inoculated with the brain matter of each of them, some intra-peritoneally, others subcutaneously; furthermore, the entire brain matter, lungs, liver, and spleen of the two sacrificed animals was reduced to a pulp and this was cultured on agar. By the third or fourth day, all the agar tubes were covered with two of the most characteristic gonidial forms of the pathogenic mold: the *Penicilium* form and the *Mucor* form.

The different types of the *Penicilium* form are shown in Figures 1 to 11 of Plate 227. This form is distinct enough to make identification of the mold easy.

Photographs of the different types of the *Mucor* form are contained in Figures 1, 2, 3, and 4 of Plate 228. This *Mucor* form is very distinctive and has a very particular appearance that makes it easily recognizable. Dr. Bialomskja had warned me that the disease is most reliably reproduced by inoculation with the brain matter of the inoculated animals. In fact, the pulp of this brain matter, spread in a thin layer on agar and stored at 22-24°C, becomes completely covered by the *Mucor* form on the third day of cultivation. It is a mold 1 centimeter or more high, of a dirty yellowish cream or dirty grayish ochre color. When replanted, or in its original cultures, this *Mucor* form almost always spontaneously gives rise to the *Penicilium* form.

It is easy to ascertain, by mid-examination microscopically, that this spontaneous transformation of the *Mucor* form into *Penicilium* occurs by direct development, on the *Mucor* filaments, of mycelial filaments bearing the characteristic fruiting bodies of the *Penicilium* form (Figs. 5, 6, 7, plate 228). The transformation is therefore total from the outset and does not include intermediate forms.

The cultures also show, when carefully examined microscopically, a transformation of the *Mucor* type into a *Monosterigmata* gonidial form.

This form is represented in Figures 10, 11, Plate 228.

Finally, in the pulp cultures of the lung tissue, a fourth gonidial form has developed; the *Sterigmatocystis* form represented in Figures 12, 13, Plate 227.

On the cultures of the pulp of the organs of the inoculated animals, a soft, creamy material develops, apart from the gonidial forms of the pathogenic mold, and flows slowly to the bottom of the tubes. This material is the bacterial culture of the pathogenic agent represented by the photographs in Figures 8, 9, 18, Plate 228; this fact is confirmed by the formation of the same creamy material, containing the same bacterial elements, in the pure, subcultured cultures of *Mucor*; Figure 12 contains a photograph of the bacterial forms thus developed in a pure culture of *Mucor.*

These cultures contain long bacterial elements that segment into smaller elements forming the majority in the cultures; this segmentation gives rise to coccobacilli equipped with bipolar globules, which are the characteristic element of the cultures; they exist in Figures 8, 9, 12, 13, but are most clearly distinct in Figure 13.

The bacterial culture, as well as the *Mucor* and *Penicilium* forms of the pathogenic mold, possess the same virulence as the brain pulp of animals carrying typhus and kill the guinea pig just as quickly, perhaps faster.

Identification of the pathogenic mold and search for the source of the virus.

The gonidial forms of the typhus mold were easy to identify because of their distinct shape. These are the gonidial forms of the organic mold of oats.

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The *Penicilium* form of oats is represented in figures 1 to 7 of plate 229, and figure 9, plate 160. Comparison of these figures with those of plate 227 (exanthematic typhus) shows perfect identity.

This *Penicilium* form of the organic oat mold was obtained by culturing on agar the pulp of the organs of a guinea pig inoculated with the spores of *Ustilago Aveni*; it is obtained identically by inoculating an emulsion of healthy grains or crushed aseptic leaves; it is also obtained identically by direct culture on agar of aseptic oat leaf pulp.

The *Monosterigmata* gonid form of oats (Fig. 2, pl. 160), which is very characteristic and whose confusion with another is impossible, is clearly identical to that of the exanthematic typhus mold (Fig. 10, 11, pl. 228).

The bacterial culture of organic oat mold is also identical to the bacterial culture of the exanthematic typhus agent. It is easily obtained by inoculating broth with an emulsion of crushed oat grains or with the pulp of young crushed leaves, obtained in a few days by aseptic cultivation of oat grains in sterile moist sand. When cultures are grown on agar, the same creamy material develops on the surface as in pulp cultures of inoculated animal organs.

The bacterial elements are the same in the two orders of culture; compare in this respect figure 12 (exanthematous typhus) with figure 8 (oats). The characteristic element of the bacterial cultures of the organic mold of oats is a coccobacillus provided with bipolar corpuscles identical to the coccobacillus reported in the bacterial cultures of exanthematic typhus.

This identity of the three characteristic elements: *Penicilium* form, *Monosterigmata* form, form of bacterial elements, would suffice to establish that the pathogenic mold which is the agent of exanthematic typhus is the organic mold of oats.

In a first series of studies aimed at searching for the various gonid forms of the organic mold of oats and fixing them through photography, I did not succeed in obtaining the *Mucor* form; it was obtained in a new research carried out with a view to an additional check of the accuracy of the previous conclusion; it is perfectly identical to the *Mucor* form of exanthematous typhus; it is therefore certain that, in new attempts, we will

obtain, by direct or indirect transformation of the *Penicilium, Mucor, Monosterigmata*, or *Sterigmatocystis* forms of typhus, the *Aspergillus* form of the *Fumigatus* type, which is a gonidial form of the organic mold of oats.

From these facts, I conclude that:

The pathogenic mold that causes typhus is the organic mold of oats or one of its gonidial forms (Mucor).

The original sources of the virus are therefore:

1. The rotting of oat straw in manures and the liquid manure that flows from them.

2. The bacterial elements developed in the soil by the roots in oat fields and their transport by rainwater.

3. Spores and zymospores of oat smut (*Ustilago aveni*) introduced into homes with sheaves of oats and spread through the air and onto the ground during threshing.

4. Oat grains fed to animals (poultry, horses) and rotting on the ground.

5. Oat flour diluted in water and left to remain there.

6. Horse dung.

7. Food contaminated with manure, especially milk.

Oats being one of the most cultivated plants and the straw of this plant being one of the main components of manure in all countrysides and in all countries, it is certain that the typhus virus is one of the most widespread in all countries The fact that this disease has almost completely disappeared in certain countries, despite the almost constant soiling of the ground, the floors of houses, the clothes and the hands of rural inhabitants, shows that the infection is extremely rare; the coincidence between the disappearance of the disease and the development of care for the cleanliness of the body in these countries shows that the condition of the infection is uncleanliness and notably the constant soiling of the body by manure combined with the proliferation of lice on the body. Lice, infected by manure, like the body which hosts them, inoculate by their bite the bacterial culture of the organic mold of oats. These facts explain the primitive inoculation of typhus; lice, as we know (and perhaps also mosquitoes) cause its spread by their passage from contaminated individuals to healthy individuals.

EPIDEMIC DYSENTERY

The following study relates to two types of epidemic dysentery bacilli: *Shiga* type and *Flexner* type, cultures of which were made available to me by Professor Legroux of the Pasteur Institute. The transformation of these two bacillary types into hyphomyoetes is easily obtained by subcutaneous inoculation of the guinea pig and culture, on agar, of the pulp of its organs, lung, liver, spleen, etc. The *Penicilium* form appears in the cultures around the fourth day.

Figures 1 to 8 of Plate 230 represent the various aspects of the *Penicilium* form of the bacterial type designated *Flexner* 2; Figures 9 to 15, the aspects of the *Penicilium* form of the *Shiga* type culture.

Identification and original source of the virus.

During studies to identify smallpox mold, I investigated the gonidial and bacterial forms of organic mold in a number of food plants. The pathogenic action of the bacterial cultures obtained was studied on rabbits; I noted that bacterial cultures of species of the genus *Brassica* caused diarrhea, sometimes bloody, in the inoculated animals. The *Penicilium* form of these species that had been isolated was compared to the *Penicilium* forms obtained by transformation of cultures of dysentery bacillus. This comparison led to the conclusion that they were specific. The *Penicilium* forms of *Brassica* species and their varieties are very similar, so it is difficult, by such a comparison, to decide with certainty, for example, whether the *Penicilium* form resulting from the transformation of the *Shiga bacillus* corresponds to the *Penicilium* form of the turnip (*Brassica campestris,* var. *rapifera*), to that of the turnip cabbage (*Brassica napus, var. napobrassica*), or to that of one of the varieties of *Brassica oleracea* (headed cabbage, Brussels sprouts, cauliflower, kohlrabi, etc.). Plate 231, given here as an example, contains a series of *Penicilium* forms of the organic mold of the turnip (*Brassica campestris,* var. *rapijera*), a careful examination of which will show the close relationships with Figures 9 to 15 of Plate 230 (*Shiga*).

A more precise determination can only result from a more extensive study of the various gonidian forms already isolated and photographed and of the bacterial forms of the organic mold of the different species and varieties of the genus *Brassica* (cabbage).

Bacterial cultures of the different species or varieties of the genus Brassica are easily obtained by inoculating the central part of the stem of these various plants, reduced to a pulp, into broth or on agar. The agar cultures give rise to a creamy, often semi-liquid

material, flowing to the bottom of the tubes, which is the bacterial culture of the plant. The study of these cultures and the comparison of their elements with those of the dysentery bacilli reveals an identity similar to that already observed between the *Penicilium* forms.

The bacterial elements of a culture from green cabbage (*Brassica oleracea*, var. *acephala*), for example (Fig. 11, pl. 232), are almost identical to those of the culture from another species, the turnip (*Brassica campestris*, var. *rapijera*) (Figs. 9, 10, pl. 232). Both cultures show polymorphic elements exhibiting the morphological characters of dysentery bacilli; they contain bacillary elements 2 to 3 u long and shorter elements; some are provided with bipolar globules; the width of the elements is also variable; there are almost filiform elements; these characters can exist as early as the second day; but for certain species, the shape and appearance of the bacterial elements varies with the age of the culture For the turnip, for example (*Brassica campestris*, var. *rapifera*), the cultures contain, on the second day, larger bacillary elements than those that form later; they are 3 µm long by 0 µm. 4 µm wide and segment into two shorter, ovoid bacilli, which remain attached end to end and give a characteristic appearance to the culture (Fig. 10, plate 232). Some of these elements are found in older cultures, mixed with finer bacillary forms.

* * *

Plate 232 also contains photographs of the bacterial forms of other food plants; Figures 1 to 6 show the bacterial forms of the organic mold of curly endive (*Cichorium endivia,* var. *crispa*), and provide an example of the polymorphism of cultures and the variation of forms with age (see the leg-end to the figures).

Figures 7 and 8 show the bacillary forms of organic radish mold (*Raphanus salivus*, var. *radicula*) in two-day-old (Fig. 8) and four-day-old (Fig. 7) cultures.

Bacterial cultures of other plant species frequently cause diarrhea, sometimes bloody, when inoculated into animals; therefore, it is likely that bacterial cultures of plant species other than those of the genus *Brassica* can cause epidemic dysentery. Bacterial cultures of various plant species can be characterized fairly easily and most often have characteristic forms.

Crops are generally very similar when they are apparently very different plants, but belonging to the same species or to related species. This is true, for example, for varieties of *Brassica oleracea* (kohlrabi, Brussels sprouts, headed cabbage, cauliflower) and even for related species (*Brassica oleracea* and *Brassica campestris*). Plate 233 provides another example in edible red beet (Figs. 1 to 8) and *Swiss chard* (Figs. 9, 10, 11), which

are both, despite their apparent difference, varieties of the same plant species: *Beta vulgaris*.

On the other hand, bacterial cultures of species belonging to different families become clearly distinct. Plate 33 gives an example of this in Figures 12, 13, which represent the bacterial forms of the organic mold of leek (*Aillum porrum, Liliaceae*) in a four-day (Fig. 12) and twelve-day (Fig. 13) culture; for this plant, the bacterial cultures are characterized by a spindle-shaped bacillus provided with bipolar globules.

We can thus judge, in plates 232 and 233, the variability of bacterial forms in very dif - ferent plant families: *Cruciferae* (turnip, fig. 9, 10; cabbage, fig. 11; radish, fig. 7, 8, pl. 232); *Compositae* (chicory, fig. 1 to 6, pl. 232); *Chenopodiaceae* (edible beetroot, fig. 1 to 8; chard, fig. 9, 10, 11, pl. 233); *Liliaceae* (leek, fig. 12, 13, pl. 233).

This presentation helps us understand why epidemic dysentery can be caused by different bacillary types, morphologically similar, originating from a group of very similar food plants belonging to the same genus or species (*Brassica*); this group of food plants easily gives rise to bacterial cultures, either by putrefaction of plant parts rejected and abandoned on the ground or in manure, or by fermentation developed for food purposes (sauerkraut), or by *spontaneous* fermentation of cooked foods in which the vegetative elements, particularly the zymospores, are not destroyed by boiling. These same bacterial cultures can also develop after ingestion of raw foods whose digestion is incomplete or difficult: raw cabbage salad for example.

Antrax

The direct transformation of a smut (charcoal) culture into a hyphomycete is obtained by transferring it to solid media: cooked meat, casein, egg yolk, carrot, etc. In a first attempt, I obtained the transformation of a culture into the *Penicilium* form (Fig. 3, 4, 5, pl. 237).

Subsequently, the same culture spontaneously transformed, on carrots, into the *Aspergillus* form (Fig. 1, 2, pl. 237). These transformations were obtained at the very beginning of the research that is the subject of this work; wanting to verify whether the *Aspergillus* and *Penicilium* forms do indeed have a specific link between them, the spores of the Aspergillus form were inoculated into the guinea pig; after the death of the animal, the pulp of the organs (lung, liver, spleen), cultivated on agar, gave rise to the same *Penicilium* form (Fig. 1, 2, 3, pl. 238) as that previously obtained by direct transformation; I also noted that the mycelium of the culture obtained emitted fine filaments that segmented into anthrax bacilli (Fig. 3, pl. 238).

This observation prompted me to try to bring back, by direct transformation, the *Aspergillus* form of anthrax into bacterial culture.

The inoculation of the spores in glycosylated peptone calf broth gave rise to the desired culture. The spores, or the first elements they form, gather in clusters which constitute germinating masses. These develop one or more large mycelial branches (Fig. 2, plate 239) which ramify and laterally emit fine filaments whose segmentation constitutes the anthrax bacilli; Figure 3, plate 239, shows these very distinct filaments, emanating from the primitive mycelial trunk, and dividing into characteristic bacilli; This figure, showing the germinative mass, the primitive mycelial trunk, its secondary branches and their segmentation into bacilli, constitutes the exact picture of the general mode of formation of bacilli and of the cycle of evolution of the bacterial elements; the bacilli segment themselves into smaller elements, until the formation of the granulation having as a diameter the width of the bacillus. These granulations then come together in clusters of 3, 4, 5, or more and are agglutinated together by a hyaline matter; the mass, enlarged by the addition of new basophilic granules, thus comes to constitute a germinative mass. Figure 3, plate 239, shows these germinative masses, *identical, when they are formed, to leukocytes* seen in all stages of their development.

In a normal anthrax broth culture, which has not undergone any transformation, the same elements are present, as demonstrated in Figure 1, which is a photograph of a fourteen-hour-old anthrax broth culture; in this same figure, we see, on the right, a germinative mass emitting its mycelial branch, which will be the origin of the filaments whose segmentation will give rise to the bacilli.

In the germinative masses in formation or constituted, we observe the presence of non-basophilic refractile granules; I have not been able to determine whether these granules are emitted directly by the mycelial filaments or whether they are spores formed on spore-bearing hyphae.

The clear, refractile spaces that exist in Davaine's bacteridium and which have been considered the anthrax spore appear to be formed only by the retraction of the basophilic protoplasm at the points of future segmentation of the bacillus evolving towards the granular form. The characteristic shape of the bacilli, in cultures obtained by transformation of the *Aspergillus* form, seems to indicate that anthrax is caused by the bacterial vegetation of this gonidial form.

Like other bacterial cultures, the filtered anthrax culture contains the zymospores (or toxin) of the anthrax mold. I attempted to reproduce the mold with these zymospores alone; I obtained it in the form of a white, powdery culture, chalky in color and appea-

rance. It is formed of very fine, tangled filaments emitting spores in a string from their ends and does not possess a characterized gonidial form.

This same white, chalky mold grows on old cultures of the *Aspergillus* or *Penicilium* form of anthrax, or on the surface of the broth deposited at the bottom of carrot culture tubes.

Identification of anthrax mold has not yet been achieved.

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CHOLERA

I studied the cholera pathogen using three cultures, one designated Pot cholera, another Bologna vibrio, and the third Jaritzin vibrio, made available to me by Professor Legroux of the Pasteur Institute.

The first culture is one of the first from which I succeeded in obtaining hyphomycete forms. Agar cultures were scraped with a spatula and spread on various solid media; the *Penicilium* form of the cholera mold developed after three or four weeks on two of the media: on the one hand, on egg yolk (Figs. 1, 2, 3, 4, pl. 234); on the other, on fire-clay soaked in broth (Figs. 5, 6, 7, pl. 234).

Since direct transformation processes are lengthy, the hyphomycete forms of other cultures were sought by indirect means: inoculation of the guinea pig and culture of the pulp of its organs on agar; the cholera mold develops from the third to the fourth day.

With the cholera culture designated Bologna vibrio, I obtained the cholera mold in two gonidian forms: the *Penicilium* form and the *Mucor* form. The mycelium of the *Penici - lium* form, represented in the figures on plate 235, often has a characteristic peculiarity; it is strongly undulated and appears (Figs. 1 and 2, plate 235) to be twisted into a spiral whose turns are widely separated from each other. It is in fact the segmentation of fine spiral or undulated filaments which, in bacterial cultures or in the organism, gives rise to the arched, or comma-shaped, forms of the cholera vibrio. The *Mucor* form of the cholera vibrio is represented in figures 1 and 2 of plate 236; it is of the *Mucor racemosus* type.

With the culture designated Jaritzin's vibrio, I obtained the cholera mold in the gonidial forms *Penicilium* (Fig. 3, 4, pl. 236), *Sterigmatocystis* (Fig. 6), *Aspergillus* (Fig. 6, 7, 8, 9, 10), and *Mucor.*

Identification of the cholera mold.

It is known that the death cap and citrine amanitas cause poisoning, which has been attributed to a toxic ferment called amanitin; this ferment is not destroyed by boiling.

I have studied this poisoning by the death cap and citrine amanitas at some length. The physiological phenomena that occur are *in all respects identical to those of anaphylactic shock;* there is a very notable lowering of blood pressure, a considerable decrease in the coefficient of blood irrigation (collapsed veins), and a decrease in the glycolytic power of the blood. The hypothermia, which accompanies the poisoning, is due to the decrease in intraorganic combustion.

These symptoms, as we know, bear a striking resemblance to those of cholera.

Since the death cap and citrine amanitas are made up of an organized mold, the toxic ferment they contain is made up of their zymospores. They resist boiling and retain their toxic power despite it.

Since the original source of cholera mold is necessarily the organic mold of an animal or plant, and the symptoms of cholera have a known identity with those of poisoning by death cap and citrine amanitas, it was therefore advisable to investigate whether the living beings, which are the original source of cholera molds and their bacterial culture, are not these fungi.

To this end, the gonidial forms of the organic mold of these two species of fungi were sought, either by direct culture on agar of the aseptic pulp of the stipe and cap, or by culture of the organs of a guinea pig inoculated with this pulp.

I thus obtained, by these two processes, the *Penicilium* forms and the *Mucor* forms of the death cap and *citrine amanitas*. The comparison of these gonidial forms with those obtained by transformation of three cholera cultures could leave no doubt.

The various types of each cholera mold photographed in plates 234, 235, 236, being few in number (I was unaware of the possibility of identifying them when I photographed some types), I referred to the original preparations. From the comparison of a large number of types, I concluded that there was perfect identity. The *Penicilium* forms (Pl. 235 and 236) of the cultures designated vibrio of Jaritzin and vibrio of Bologna (one of the characteristic types is that of fig. 4, pl. 236) were clearly identical to the *Penicilium* form of Amanita citrina; we observe, in the preparations of *Penicilium* of Vibrio of Jaritzin, for example, all the types of *Penicilium* of Amanita citrina; moreover, the *Mucor* form of the latter was completely identified with the form *Mucor* of the Bologna vibrio (Fig. 1, 2, pl. 236).

There was a very certain identification here; it was all the more so since certain characteristic features of the *Mucor* form of the Bologna vibrio, the presence of sessile spherical fructifications in the form of warts (spores or chlamydospores) on the mycelium were faithfully reproduced on the mycelial branches of the *Mucor* form of *Amanita citrina.*

The types of *Penicilium* obtained from the cholera culture from Professor Bezançon's laboratory (designated Cholera Pot, pl. 234) were more closely related to the *Penicilium* form of the death cap (Pl. 326). Later, many more types of vibrio cultures were photographed, but too late to be included in this volume; they will be included in another publication. Currently, the identification of vibrios from other sources is being sought.

Characteristics of cholera vibrio cultures.

It is known that the arched shape is in no way characteristic of the cholera vibrio. Other species exhibit the same shape. Moreover, in cultures, straight forms are found alongside arched forms; in some, arched forms are rare and almost all the bacillary elements are straight.

From various treatises on Bacteriology, I extract, for example, the following notions:

"The various races of vibrios present very marked morphological differences among themselves, such as length, thickness, and degree of curvature; in some races, elements are observed to be so short and stocky that their shape becomes almost ovoid. Vibrios cultivated for a long time on artificial media can lose their curved shape and transform into elongated and thin rods'' (*Kolle and Hetsch, Treatise on Bacteriology,* page 180).

"There are varieties of vibrio that deviate in particular from the Koch type. Some are thin, irregularly curved, and sometimes have the shape of an elongated S (Vibrio of Massawa, of Courbevoie, of Paris). Other vibrios are rectilinear and never have any curvature (Vibrio of Shanghai); still others, very small, coccobacillary (Vibrio of Malta). Moreover, Metchnikoff, by re-inoculating an old culture in peptonized water of Vibrio of Angers, usually stocky and curved, obtained a thin and elongated shape ''
(*Microbiological Technique,* Besson, 1911).

In reality, any cholera bacillus can take the different forms indicated above and even others. A cholera vibrio can lose its arched shape by inoculating it into a guinea pig and culturing the organ pulp on agar after death; a semi-liquid material develops in the cultures in a layer or in small, round, distinct colonies, which are a bacterial culture of

the vibrio; generally, only straight forms remain in this culture. When transferred to broth, it generally only gives straight forms.

I obtained such cultures for the three vibrios of different origins that the Pasteur Institute made available to me.

If a fairly large quantity (1 to 2 cubic centimeters) of cholera vibrio broth culture is inoculated under the skin, they are found in the intestine in two forms: as thin bacilli (0.6 to 0.7 microns by 2 microns) and as thick bacilli (1 to 1.2 microns wide by 3 to 6 microns by 9 microns long). The animal does not suffer from diarrhea, but the small intestine is often found distended by a thick, yellowish porridge, rich in cholera bacilli of both sizes and in germinative masses.

Vibrio cultures change shape; a broth culture that contains many arched, V-shaped, and S-shaped forms may contain only straight bacilli after a certain number of subcultures. Other times, a culture that contains only straight bacilli forms arched bacilli as it ages. In vibrio cultures, the following elements are observed if care is taken to monitor their development by frequent examinations repeated over a period of two weeks:

1. Mycelial filaments of variable length, flexuous or spiral, or convoluted in all possible forms. The filaments have this form only in cultures containing arched forms. In cultures containing only straight bacillary forms, the filaments are barely flexuous or straight. The width of these filaments is, in general, that of the bacillary elements, that is, 0.6 to 0.7 μm. 7. In cultures containing bacilli of two different sizes, that is, 0.7 and 1.2 μm. wide, there are large unsegmented or segmenting mycelial filaments, 1.2 μm. wide or slightly less.

2. Straight bacillary elements with rounded ends, 0 μ. 6 to 0 μ. 7 wide and of variable length. The long mycelial filaments always segment into elements 2 to 3 μ long. The most common dimension is 2 μ. Bacilli of 2 μ segment into two coccobacilli 1 μ long; and bacilli of 2 1/2 to 3 μ, into short bacilli of 1 1/4 to 1 1/2 μ.

Coccobacilli of 1 μ segment themselves into two round granules and those of 1 1/4 to 1 1/2 μ into oval granules.

Some bacilli lose their basophilic protoplasm, which they release into the culture medium in the form of granules (zymospores); they thus become poor in basophilic protoplasm and poorly stained with basic colors. They may retain two grains of basophilic protoplasm in the form of globules or polar spots.

3. Straight, thick bacillary elements with rounded ends, 1 μ to 1 μ.2 wide, some- times even more, by about 3 μ, 6 μ to 9 μ long. 3 μ bacilli segment, forming two large coccobacilli of about 1 μ.5.

Sometimes, segmentation occurs differently; a large bacillus of 3 µm, for example, gradually loses part of its basophilic protoplasm in the form of granules (zymospores) which are released into the liquid medium. The bacillus then displays four or five transverse segmentation lines, then fragments into five or six granules poor in basophilic protoplasm.

Vibrio cultures often possess only the small bacillary form; by searching properly in the cultures, however, some large elements can be found, although very rare. But the large bacillary form can be revealed by the following processes:

By subcutaneous inoculation of 1 to 2 cubic centimeters of broth culture into a guinea pig, the large bacillary form is found after death in the small and large intestines, mixed with the small form; by culturing the pulp of the liver, spleen, and lung on agar, both bacillary forms are often found in the whitish coating that flows to the bottom of the culture tubes.

If, on the other hand, an agar culture is inoculated onto various solid media, egg yolk, beef, casein, etc., *with very little moisture,* it passes very quickly into the mycelial state. We see that large mycelial filaments of 1 to 2 (and larger) are formed, which emit finer filaments. The development of these mycelial filaments of various sizes helps us under-

stand why there are thin and thick bacilli in cultures; it is because thick mycelial filaments are formed there, *on which less wide filaments are born,* and all the filaments, thick and thin, segment into bacilli. The thick bacilli have been seen by bacteriologists; they call them involution forms; Kolle **(1)** describes them as *"thick, swollen individuals, which stain with difficulty and are forms of involution or degeneration."*

If the culture on solid media is inoculated into broth when it has reached this state, it reproduces a bacterial culture containing either only large bacillary elements, or a mixture of large and thin elements.

4. Germinal masses. They are found in all stages of development; fully formed germinal masses have the appearance of either a granular element of round, oval, or irregular shape, containing a round nucleus of variable size, often very large (5 to 10 µm or even larger). Other germinal masses have the appearance of either a spherical mass 8 to 12 in diameter, strongly basophilic, which could be taken for a mononuclear leukocyte, or of an oval mass that is uniformly basophilic or contains only a basophilic nucleus. In some cultures, germinal masses are observed as early as the second day; they are almost always found in older cultures.

(1) *KOLLE and HESTCHE, Experimental Bacteriology, page 107.*

5. In old cultures, very large, irregularly shaped elements often form, in the form of spindles or double spindles stuck end to end; some elements are swollen in places, others have the shape of a cone. These are either elements of mycelial segmentation or irregularly shaped agglomerated masses.

6. Granulations of various sizes. They are animated by Brownian motion. These are the zymospores of the culture; they constitute the toxin. They come either from the bacillary elements which expel their basophilic protoplasm in the form of granules, or from fine, very tortuous, branched mycelial filaments, on which they are born directly in the form of fine, almost sessile basophilic spores; these filaments, which form zymospores, emanate either, most generally, from round or oval spores of 1 μm, or from small germinative masses of 1 to 3 or 4 μm, or even from larger masses.

They can develop even on large bacilli, which thus directly act as germinative masses. A considerable mass of these filaments develops in the intestine of guinea pigs that have received 1 to 2 cubic centimeters of a vibrio culture by subcutaneous injection.

Examined between slide and coverslip, a vibrio culture shows highly mobile bacillary elements and zymospores exhibiting lively Brownian motion. These movements were considered active and due to a vibratory cilium placed at the end of the vibrio.

These movements are not active; they are passive and due to a zymospore which, attached to a bacillus, draws it along in its movement. By following the elements in their passive movements, we can distinguish the zymospore that draws them along and perceive its own movements. Examination of the culture shows that a string of bacilli, a long mycelial filament, a germinative mass, or any element, even shapeless, is just as well drawn along as a bacillus by one or more zymospores that can be clearly distinguished.

Direct examination of the bacilli, strongly stained by dahlia, shows that they do not possess vibrating cilia. The large bacillary elements, 1 to 1.2 μm in size, often emit mycelial filaments; but they can emit them both on the sides and at the ends, and these filaments often carry several zymospores that they have formed. The filament may be smooth or wavy, but it is a germinative filament and not a cilium.

After this study of the elements of vibrio cultures, let us point out that a vibrio of a given origin can appear in several different forms depending on the treatment to which it has been subjected; if it is perpetuated on agar and cultivated at 38°, it will take on a different form than if it is preserved at room temperature and perpetuated in broth; if it has passed through the animal several times and if it has been subsequently cultivated in an artificial medium, it can develop either in the thin bacillary form, or in the double thin and thick form; it can present only straight forms or a mixture of arched

and straight forms; it can even develop only in the form of granules, without a single bacillus being found there.

The evolution of cultures is as variable as the form of the elements; the destiny of the bacilli is to form germinative masses. In certain cultures, the bacilli fragment very quickly into three or four granulations which unite to form germinative masses; in other cultures of the same vibrio, the division into granules appears not to exist and

the germinal masses are formed by the agglomeration of non-segmented bacilli; other times, in cultures with arched forms, the bacillus curls completely on itself and merges into a round mass which unites with others; or again, the end of a filament curls several times on itself in different directions to form a ball which merges into a germinative mass. In cultures with the large bacillary form which segments into large coccobacilli, these group together in clumps which agglomerate several thin or large basophilic bacilli, to form a germinative mass.

As can be seen, the shape of the elements and their evolution are extremely variable and this variation is observed for the same vibrio of known origin.

This fact is of great importance, because it shows that one cannot rely on the morphological characteristics of a single bacterial element to determine whether a germ found in a patient's stool is cholera. For this determination, it is necessary to study all the evolving forms of the bacterial element; another equally reliable check is carried out by the transformation of the germ into the *Penicilium* form of the original mold; this form is very distinct and characteristic.

* * *

We will conclude this study with a few words relating to the Pfeiffer phenomenon. It is known that vibrios, injected into the abdominal cavity of a guinea pig with serum from an immunized animal, quickly transform into granules, then disappear completely. It is said that they have been destroyed, bacteriolyzed by the serum. This description of the phenomenon is incomplete. In these experiments, the bacilli do indeed transform into granules, which themselves disappear; but, if we no longer find these granulations floating in isolation in the liquid, we find in it a new element that they have formed characteristic germinal masses.

It has been observed that leukocytes appear in the liquid; but they have been taken for normal leukocytes of the guinea pig which arrive in the liquid to phagocytose the vibrios; these leukocytes do not have this origin; there are perhaps some which belong to the guinea pig; but almost all of the leukocytes which appear are those of the culture of the vibrio and are the germinative masses formed by it. The vibrios transform into granulations, and these agglomerate into germinative masses.

To clearly demonstrate the real meaning of the phenomenon, it is necessary to distinguish between the normal leukocytes of the guinea pig and the germinative masses resulting from the transformation of the bacterial elements of the lemon and death cap mushrooms, with which they bear a certain resemblance. This distinction was easily made. I photographed the germinative masses of the in vitro cultures of the lemon and death cap mushrooms and the stages of their formation. I thus observed that the bodies removed from the guinea pig's peritoneum are identical to the germinative masses of the in vitro culture.

Other observations allow us to state with complete certainty that the leukocytes formed are those of the culture and not those of the guinea pig. Indeed, the germinal masses or leukocytes of in vitro cultures of citrine amanita are clearly different from those of death cap cultures. However, the germinal masses removed from the peritoneum of the guinea pig that has received a bacterial culture of citrine amanita are identical to those of this in vitro culture and *very different* from those removed from the peritoneum of the guinea pig that has received a culture of death cap, and which are themselves identical to those that appear in the peritoneum.

Thus, the shapes of the leukocytes that appear in the peritoneum of the guinea pig, into which a bacterial culture is injected, *are very different depending on the species injected, and always resemble the native germ masses in vitro of that species.* If they were the leukocytes of the guinea pig, they would always be identical, regardless of the bacterial species injected. Now, not only are the leukocytes that appear in the peritoneum of the animal identical to those of the injected bacterial culture, but they are also very different from those of the guinea pig.

Another equally demonstrative proof of the origin of leukocytes is provided by the examination of the contents of the small intestine of guinea pigs that have received, by subcutaneous injection, one or two cubic centimeters of a culture of cholera vibrios or of amanita citrine or phalloides. After the death of the animal, the contents of the small intestine are spread on a slide, fixed by heat and colored with dahlia; we observe:

1. In guinea pigs inoculated with a culture of amanita citrine, germinative masses identical both to those observed in old bacterial cultures on agar in vitro, and to those

contained in the peritoneal cavity of the guinea pig one and a half hours after the injection, into this cavity, of the culture mixed with immunizing serum.

2. In guinea pigs inoculated with a culture of death cap, germinative masses identical to those observed in the peritoneum (Pfeiffer's experiment) or in in vitro cultures.

This is therefore another decisive demonstration of the origin of the germinative masses formed, because, since the inoculation of the culture was done under the skin, it could not be argued that the germinative masses (heterogeneous leukocytes) observed in the intestine had crossed the abdominal wall; this fact is impossible due to the large dimensions of the elements and, moreover, has no apparent reason to exist, since phagocytosis does not exist.

We also note that, in both cases, the intestine contains numerous straight bacilli of the two usual sizes, thin and wide, characteristic of cultures of the citrine and death cap mushrooms; it is therefore the zymospores of the culture injected into the subcutaneous connective tissue which, swarming throughout the organism, crossed the intestinal mucosa and reconstituted the bacterial culture in the intestine.

There are therefore two distinct and decisive proofs of the origin of the germinal masses formed. The germinal masses removed from the peritoneum of the guinea pigs, as well as those from the in vitro cultures, were photographed and will appear in a later publication.

It is easy to ensure, moreover, that the serum of the immunized animal only has an accelerating effect on the development of the vibrios by following this on the elements injected into the peritoneal cavity of the guinea pig with salt water instead of immunizing serum. The development of the injected culture takes place, in this case, as in Pfeiffer's experiment, *but less quickly.* I followed this development for the cultures of *Amanita citrina* and *Amanita phalloides*. For the former, if the culture injected without immune serum is removed from the abdominal cavity an hour and a quarter after the injection, it is observed that almost all the bacilli have transformed into granulations, and that these come together in large clusters to form germinative masses; many masses are already formed; only a very small number of bacilli remain, not fragmented into granules.

For the culture of the death cap, the evolution is even clearer. Having injected 2 cubic centimeters of diluted immunizing serum, added to bacterial culture (1 cc. 2 of serum at 1/250e, 0 cc. 8 of culture), I found that after an hour and a quarter there was not a

drop of liquid left in the peritoneal cavity. It was necessary to add salt water to the intestinal mass and shake it to be able to check, by examining the contents of the water, whether there were any bacilli left in the peritoneal cavity. They had almost completely disappeared.

In another experiment, a mixture of salt water (1 cc. 2) and a culture of *Amanita phallus* (0 cc. 8) was injected into the abdominal cavity. After an hour and a quarter, as in the previous experiment, no trace of liquid remained in the peritoneal cavity; salt

water being spread over the intestinal mass and the latter being agitated, I found only rare bacilli in the water, but, on the other hand, numerous germinative masses. In the case of *Amanita phallus,* the evolution of the vibrios is therefore almost as rapid when they are injected alone into the peritoneal cavity as when they are injected there with immunizing serum.

The Pfeiffer phenomenon therefore does not consist of a bacteriolysis of the vibrios, but only in an acceleration of their evolution. The effect of the serum of the immunized animal is not to destroy vibrios, but to accelerate their transformation into granulations, then their agglomeration into germinal masses; it therefore has an effect on the bacterial phase of the disease, which it shortens. The phenomenon of the transformation of vibrios into granules and their grouping into germinal masses is normal, natural, and constant for any vibrio introduced into the body, and in no way caused by the immunizing serum; this serum only accelerates this normal phenomenon.

Characteristics of the bacterial cultures of *Amanita phalloides* and *Amanita citrina*.

The characteristics of the cultures of cholera vibrios having been established, we will now study the bacterial cultures of *Amanita citrina* and *phalloides*.

A bacterial culture of these mushrooms is easily obtained by tearing the stem or cap of a young, carefully picked, uncontaminated specimen into two parts and scraping the surface of the tear with a thin spatula to obtain a semi-liquid pulp, which is inoculated into broth kept at room temperature. The next day or two, the culture becomes cloudy and vegetates.

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I will not describe the forms of the elements of these cultures, because, to characterize them, it will suffice to say that they are rigorously identical to those of vibrios of different origins isolated from the stools of cholera patients, and that everything that has just been said about the cultures of these vibrios applies entirely to the bacterial cultures of *Amanita citrina* and *Amanita phalloides*.

The citrine amanita, inoculated in broth, quite often gives rise, at the beginning, to a pure culture of the large bacillary form 1µ to1µ.2 wide; this form is so distinct from the known appearance of cholera vibrios that a bacteriologist would immediately eliminate it as being a very different species. However, if we follow this culture, we see that after fifteen to twenty days the elements segment into granulations Some of these germinate and give rise to bushy arborizations formed of fine spirillae; others gather in agglomeration masses with some of the preceding ones; then in the following days, the thin bacillary form in the form of Koch's vibrio appears in the culture. This, inoculated

at this time, immediately reproduces a culture of Koch's vibrio containing in addition some large elements, but few in number.

This culture is most characteristic; it contains, besides the characteristic segmented elements, unsegmented filaments whose particular shape, very irregular, spiral, crooked, S-shaped, U-shaped or sometimes straight, makes it clear that, depending on the position of the segmentation points, the elements formed have the shape of a comma, a semicircle, even an almost closed circle, an S, a U or a straight rod. These various forms were photographed at the very moment of segmentation alongside characteristically shaped mycelial filaments that were still unsegmented, but already bore visible segmentation features. These photographs will appear in another publication along with those of the various forms of the elements of the bacterial cultures of the sting and death cap mushrooms.

The bacillary elements of the sting and death cap mushroom cultures can take almost exclusively the form of straight, thin rods measuring approximately 0.6 μm to 0.7 μm by 2 μm; in this case, it becomes impossible to distinguish them specifically except by the shape of the germinative masses.

In in vitro cultures, it is *Amanita citrina* that is identified most perfectly, as in the gonidian forms of molds, with Koch's vibrio.

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It is therefore she that I currently consider to be the usual pathogen of cholera, although the death cap also produces cultures of cholera vibrios that are almost as virulent.

The bacillary elements of these cultures, large and thin, segment into granulations. These, especially in old cultures, germinate and emit very fine, spiral mycelial filaments whose ramifications are made up of fine spirillae that are poorly stained by basic colors. Considerable masses of these arborizations are thus formed.

When the cultures vegetate in the animal body, this germination of granulations occurs extremely rapidly; in the peritoneum of the guinea pig, there is already a considerable mass of these spirillary arborizations one hour after injection; the germinative masses of all sizes themselves emit them.

Due to the extreme rapidity of development of these arborizations, which encompass all the elements of the culture in a dense network, it appears very clearly that, when they develop in the blood, they form large clusters in which the zymospores and probably a large number of red blood cells are included. These clusters, which monopolize the zymospores and cannot possibly cross the capillaries, are obviously the direct cause of hypothermia, cyanosis, and the lowering of blood pressure in cholera patients.

These phenomena, which, like those of acute intoxication or rather of direct contamination by poisonous amanitas, are identical to those of anaphylactic shock, are therefore caused by the same cause that determines the latter: the agglutination of normal zymospores in the blood.

In acute poisoning by amanitas, it is the zymospores of the mushroom, not destroyed by boiling, which penetrate the blood, vegetate there and cause the phenomena characteristic of acute cholera **(1)**.

I also obtained bacterial cultures from other species of amanitas, notably *Amanita muscaria* (whose elements are very similar to those of *Amanita phalloides* and *Amanita caesarea*).

It is obvious that Amanita verna and Amanita pantherina, which are very poisonous, also produce very virulent bacterial cultures whose elements must be included in the series of cholera vibrios. Edible amanitas must also produce virulent cultures.

Cultures of *Amanita citrina* and *phalloides* possess the following characteristics:

1. After a few days, a viscous material like egg white often forms, which falls to the bottom of the culture. The viscous material formed in *Amanita citrina* cultures also contains yellowish lumps.

2. Agar cultures, inoculated by touching with the end of a blunt-tipped thread, have the same appearance as cholera vibrio cultures.

3. Like cholera vibrios, the elements of these cultures do not take Gram.

4. They are extremely virulent. In subcutaneous inoculation, they have the same virulence as cholera vibrio cultures and kill the guinea pig in about ten days. In intraperitoneal inoculation, their virulence is variable and difficult to fix, as is that of cholera vibrios perpetuated for a long time on artificial media; I have not investigated this virulence on cultures of recent origin.

The agglutinability characteristics of these cultures are under study; they require more careful research, which is only just beginning and cannot be discussed here.

(1) *This mechanism of amanita poisoning prompted me to examine the composition of venoms and research their mode of action. Viper venom is a liquid loaded with zymospores of the animal's organic mold. Inoculated in broth, it produces a bacterial culture. It is therefore likely solely through their vegetative elements, the zymospores, that venoms act on animals. They are viruses and not liquids acting through toxic chemical substances; inoculated, they act like a filtering virus or, more simply, like a filtered bacterial culture (toxin); the elements of these viruses develop in the inoculated organism a bacterial culture of the organic mold of the animal carrying the venom. This subject, which is under study, will be developed in a later publication.*

But whatever their results, the perfect identity of the elements and characteristics of the cultures of *Amanita citrina* and *Amanita phalloides* with those of the cultures of cholera vibrios leads to the conclusion that these two fungi are the original sources of the two most virulent agents of cholera.

From the study carried out, it appears that it is the bacterial culture of *Amanita citrina* which achieves the most perfect identity with that of Koch's vibrio, that of *Amanita phalloides* deviating from it so little that only the determination of the gonidial forms can allow the cultures to be differentiated with certainty.

If we add to this identity the fact that the cholera vibrios could be transformed into their gonidial forms *Penicilium* and *Mucor*, which are very special and characteristic, and are those of *Amanita citrina* and *Amanita phalloides*, we conclude with certainty, without any possible doubt, *that the bacterial cultures of cholera vibrio or Koch's vibrio, are those of Amanita citrina and Amanita phalloides, and that these two mushrooms are the original sources of cholera.*

They are not the only ones; other species of poisonous amanitas, and even edible amanitas, provide bacterial cultures that have similar pathogenic properties.

But among the poisonous amanitas, only the citrine, phalloides, and muscaria amanitas can be considered the original source of cholera, due to their considerable proliferation in certain years. The other poisonous amanitas, *Amanita verna*, *Amanita pantherina, Amanita virosa, Amanita alba, Amanita cariosa,* etc., can hardly constitute a notable danger of cholera infection due to the very small number of individuals that develop. On the other hand, the edible species, which develop in considerable quantities, develop a pathogenic bacterial culture as they rot: in this group, we can cite *Amanita rubescens, Amanita spissa, Amanita junquillea, Amanita vaginata,* etc.

This study finally leads us to another conclusion: *poisoning by poisonous amanitas, particularly Amanita citrina and phalloides, is a hyperacute cholera infection, caused solely by the zymospores of these mushrooms, which are not destroyed by boiling.*

It is therefore likely that the stools of individuals who died following ingestion of these amanitas contain reproductive elements of cholera vibrio cultures and are capable of contaminating other individuals.

Causes of the Development of Cholera Epidemics.

It is now clearly established that cholera develops in individuals through the ingestion of contaminated water.

A localized epidemic at the Nietleben lunatic asylum in 1892-1893 was caused by contamination of the drinking water pipe; the deteriorated filters allowed water from the Saale to pass through, in which cholera vibrios were found.

In August-November 1892, the city of Hamburg was struck by a very violent cholera epidemic (more than a thousand cases per day at one point), while the city of Altona remained virtually unaffected, as only a few sporadic cases developed there.

However, no boundary separates the two cities, which are part of the same urban area and are so closely linked that, frequently, the same street beginning in Hamburg ends in Altona.

The epidemic was explained by the fact that the two cities were supplied by two separate drinking water pipes. The one supplying Hamburg was unfiltered water from the Elbe; the one supplying Altona was water from the same source, but filtered.

It was also demonstrated that the 1892 epidemic in the suburbs of Paris was of waterborne origin.

There are quite a few cases of localized epidemics for which the importation of cholera from an infected region could not be proven. This importation was believed to be purely hypothetical:

1. Because it is known that the pathogenic agent of cholera is bacterial in nature.

2. Because it has been accepted that the original source of cholera is India and that it always comes (transported by ships and by man) either from this region or from another Asian region (or from Egypt), itself contaminated by the original source.

Determining the nature of the cholera pathogen and its original source shows that this explanation of the origin of epidemics is not exact; this pathogen is the bacterial culture of the *citrine* and *death cap* mushrooms. These two mushrooms abound in the woods throughout Europe; their natural development results, after eight to fifteen days

of growth, in rot; the stem and cap liquefy and collapse to the ground, forming a viscous mass of a nauseating odor, which allows the bacterial culture it develops to flow

into the soil. If there is a torrential rain, this bacterial culture and even the mass of this rot are carried with the rainwater into the rivers.

This source of water contamination can become very intense due to the considerable number of individuals of this species that swarm in the woods. The contamination is serious because the bacterial culture is not very demanding regarding the qualities of the culture medium and develops easily in water containing a little organic matter, which is the case for water that flows over the ground and for river water.

It should be noted, moreover, that the proliferation of citrine and death cap mushrooms (like other mushrooms) is very irregular and varies each year. In 1921, I noticed a very pronounced proliferation of amanitas in the Doubs department. In 1922, there were fewer; in 1923 and 1924 they became rare and were even completely absent in certain woods where, in 1921, one could have counted, in certain places, at least one or two individuals and even more, per square meter.

There are therefore years of great proliferation of amanitas; I believe that these are especially the years in which the summer presents successive periods of heat and rain, and which follow an already rainy year. The multiplication of the citrine and death cap amanitas, already notable the first year, becomes considerable the following year.

The bacterial culture of the citrine and death cap mushrooms, which always develops naturally on the ground when these mushrooms rot and collapse, is therefore carried by rainwater, particularly by *rainwater that flows from steep hillsides without penetrating the soil.* On hillsides, the rot of the amanitas and their bacterial culture are therefore carried directly into the rivers. *This transportation can occur within the space of an hour, during torrential rain or a storm; the contamination of the water is very noticeable if this torrential rain occurs during the amanitas' development period;* it becomes considerable and exceptional if this rain occurs in a year when the proliferation of amanitas is also exceptional. Let us add that the rain, which carries the bacterial culture of the amanitas, *accelerates their rot at the same time.*

The curves of the development of the Hamburg epidemic show that, beginning on August 16, 1892, it reached its maximum from August 26 to September 1st, then gradually declined during the month of September to end around October 15th; there

were only a few rare cases from October 15th to November 15th, while from August 25th to September 3rd there were 900 to 1000 cases per day. *The period from August 15th to September 15th corresponds precisely, for a warm and humid year, to that of the development of the citrine and death cap amanitas;* this development continues more weakly until October 1st; from then until October 15th, only rare individuals are encountered; from October 15th and after November 1st, they disappear.

It would therefore be interesting to know for the Hamburg epidemic:

1. Whether there was a proliferation of citrine and death cap amanitas in the months of August and September 1892 in the wooded regions of the Elbe valley and around Hamburg.

2. If there was a torrential rain or a series of rains in the Elbe Valley, and particularly in the lower region, in the period between August 8 or 10 and August 16, 1892.

It is likely that these two conditions must have been met, *since the bacterial culture of the cholera vibrio is that of the citrine and death cap mushrooms, and these two fungi, the original sources of the cholera vibrio, surely existed in the Elbe Valley and around Hamburg at the time of the epidemic.*

It has been thought that the Hamburg epidemic could have been due to the ebbing of the tide in the Elbe, carrying contaminated water from the port above the water intake supplying the city, or to the contamination of the Elbe by infected boatmen going up the river.

Now, the contamination of a river does not go upstream; on the contrary, it follows this current. When a river is contaminated, the infection does not come from below; it always comes from above; the cholera vibrios observed in the Elbe or in the Saale (the Nietleben epidemic) must have come from a higher region where no cases of cholera had previously been observed. On the other hand, a river cannot be contaminated by a few men; being contaminated at one point, *this point is no longer so after a few hours, because the culture follows the current of the water;* there cannot be many contaminated men because, in that case, there would necessarily be proven cases of cholera.

The proof of these assertions is, moreover, contained in the fact that, in the region of the upper course of a river, the epidemic can rage with a much greater intensity than in

the maritime region of its mouth. Thus, in the epidemics of 1873 and 1894 in the Vistula valley, there were:

For the 1873 epidemic, 1,271 cases in Kiela and 1,000 in Radom, in the upper Vistula region, while there were only 248 in Plosk (middle course), 374 in Danzig, and 564 in Königsberg.

For the 1894 epidemic, 470 cases in Kiela, 415 in Radom (upper region), compared to 190 cases in Plotsk, 40 in Bromberg (middle region), 14 cases in Danzig, and 4 in Königsberg (maritime region).

It appears that the upper region of a river is more seriously contaminated than the lower region because it is more wooded and mountainous, that is, richer in amanitas,

and better than the lower region achieves the conditions for the transmission of rot by rainwater that descends directly into the rivers without penetrating the soil, that is, without undergoing filtration.

The bacterial elements follow the course of the river, one might say, and should contaminate the inhabitants of the lower course as well. One can respond that the culture is much more virulent and richer near its point of emission and that, in river water, it does not undergo an evolution comparable to that of a culture in broth.

Whatever these considerations may be, the following facts are sufficient to explain the development of a cholera epidemic:

1. The cholera pathogen, which appears in the form of the cholera vibrio and other forms, is the bacterial culture of the organic mold of poisonous amanitas, notably *Amanita citrina* and *Amanita phalloides.*

2. The citrine and phalloides amanitas, which are the original source of the cholera pathogen, are two common mushrooms, very common in woods throughout Europe (and other continents).

3. The vegetation of these mushrooms is irregular. There are years of considerable proliferation.

4. These mushrooms rot naturally and thus produce a bacterial culture of the pathogen.

5. Torrential rains wash this bacterial culture directly into rivers, without filtering through the soil.

6. The period of proliferation of the citrine and death cap mushrooms extends from approximately August 1 (sometimes from July 1 for the citrine) to October 1. The development of *primitive* cholera epidemics, not attributable to a duly demonstrated human contagion, takes place at the same time.

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From these facts, it must be remembered from a practical point of view:

1. *That the pathogenic agent of cholera (cholera vibrio) is the bacterial culture of poisonous amanitas.*

2. That the original source of this disease exists in Europe, in all forests, these all commonly containing the death cap and citrine amanitas.

3. That the determining cause of epidemics is the violent, too frequent rain, which brings the bacterial culture of amanitas into rivers or into channeled drinking water.

4. *That, consequently, it is in years when there is a coincidence of violent and prolonged rains with a proliferation of citrine and death cap mushrooms that there will be reason to fear the development of cholera epidemics.* It will therefore be necessary, during the months of July, August, and September of these years, to exercise careful surveillance on the proliferation of amanitas, on drinking water and on river water, and, for greater safety, to use only boiled water, even with bleach added, since the zymospores of amanitas are not destroyed by boiling alone.

* * *

These causes of epidemics, determined for cholera, apply to other diseases that are currently unknown to us or whose cause is not determined. There are other species of poisonous, dubious, and even edible mushrooms that swarm in considerable quantities in woods and meadows and that produce pathogenic bacterial cultures as they rot; among these species, we can cite those of the genera *Boletus*, *Cantharellus, Collybia, Cortinarius*, etc., and especially those of the genera *Russula* and *Lactarius.* Certain mushrooms swarm at times with such abundance that it will be necessary to investigate what diseases they can transmit to humans; among them, *Lactarius piperatus, vellereus,* and *zonarius, Russula delica, nigricans, fætens, emetica*, *Hebeloma crustuliniforme*, etc.

I have already isolated bacterial cultures and gonidial forms of organic mold from many of these fungi; this study is continuing.

PLAGUE

I studied the transformations of cultures of the plague pathogen using various cultures made available to me by Professor Legroux of the Pasteur Institute, Professor Teissier, and Professor Bezançon.

The transformation of cultures into the gonidial form *Penicilium* of the plague mold is very easily achieved by inoculating them into a guinea pig and culturing the pulp of the organs (lung, liver, spleen) on agar; the mold appears on the third or fourth day.

I obtained the direct transformation of a plague culture on agar (designated Pecha plague, Pasteur Institute) by transporting it to solid media; the *Penicilium* form developed spontaneously on egg yolk (Figs. 4 and 6, plate 240). Figures 1, 2, 3, and 5 of the same plate represent other types of the *Penicilium* form from the same culture, but obtained by inoculation into a guinea pig and culture of the pulp of its organs.

Figures 7, 8, and 9 of plate 240, and 1 to 7 of plate 241, represent the various types of the *Penicilium* form from another plague culture *(Marseille plague,* Pasteur Institute);

this form was obtained by the indirect route, inoculation into a guinea pig and culture of the pulp of the organs.

Identification of the gonidial forms of the pathogenic mold.

Having isolated the gonidial forms of the organic mold from most of the most common mammals, I was struck, upon obtaining those from the rat **(1)**, by the resemblance of the *Penicilium* form to that of the plague mold; a more in-depth analysis demonstrated a complete identity.

This is not a *Penicilium* form that can be found in certain rats, but the one produced by all animals of this species, without exception.

Various types of the *Penicilium* form of the organic mold of the rat are contained in plate 242. Studying successively all the types of this plate and comparing them with those of the *Penicilium* form of the plague mold, we will note the identity which has just been noted and which continues down to the elementary forms (Fig. 2 and 5, plate 240, plague; Fig. 1, plate 242, rat).

From this observation, it resulted that the plague mold must be a vegetative form of the organic mold of the rat. Now the rat has been considered until now as the *agent of transmission* of the plague. It was therefore necessary to determine whether this animal is only *an agent of transmission or transport* of the plague virus, or whether its organism is the original source of the virus.

The rat is often infected with plague from which it dies with the characteristic lesions; but it is also known that healthy rats can be carriers of the plague bacillus or non-virulent races of this bacillus.

To verify the identification of the *Penicilium* form of the plague mold, I sought to obtain the bacterial forms of the rat's organic mold. To do this, I inoculated a piece of pulp from the spleen, liver, lung, and brain of rats (Mus decumanus) taken from the menagerie at the Jardin des Plantes into broth. In all cases, I obtained a bacterial culture: the cultures have two types in which the bacillary forms are different:

1. In the first type, all the elements have the form of coccobacilli, morphologically identical to the plague bacillus and arranged in a chain in the broth cultures. I obtained this bacillary form either by culturing the rat's brain pulp (Fig. 8, 9, pl. 241) or by culturing the liver pulp (Fig. 10, 11, pl. 241).

(1) *The gonidial forms of the organic mold of the rat were obtained by culturing the pulp of the organs of a dozen healthy animals, free from any lesion.*

2. In a second type, we find a few short chains of coccobacilli and, in addition, numerous elements having the shape of bacilli about 3 cm long, whose ends are, in general, thinned and which segment into two coccobacilli (Fig. 12, pl. 241), remaining grouped in pairs; some coccobacilli segment themselves to form two spherical granules. The coccobacilli of this type of culture, derived from long segmented bacilli, are more elongated than the chain coccobacilli.

Thus, we can obtain normal rat tissues (brain and liver, in particular), that is to say by bacterial transformation of the organic mold that from this animal, a bacterial culture of elements identical to those of the cultures of the plague bacillus.

I investigated whether the plague bacillus can present itself in the long bacillus form which exists in the second type of bacterial culture of the rat's organic mold described above. To do this, I inoculated a guinea pig with the spores of the *Penicilium* form of the plague mold isolated from an inguinal lymph node of another guinea pig which had received, under the skin of the paw on the same side, a bacterial culture of Marseille plague. On the cultures of the pulp of the guinea pig's organs, there developed, in addition to the inoculated *Penicilium* form, a creamy material which was a bacterial culture of plague; on the mold developed in the cultures, droplets of liquid containing the same culture also formed; The elements of this are represented in Figure 13, Plate 241. We notice short coccobacilli, others more elongated, in shuttle, and finally long bacilli identical to those of the bacterial cultures of the organic mold of the rat (Fig. 12, Plate 241).

There is therefore a complete morphological identity between the bacterial forms of the plague mold and the bacterial forms of the organic mold of the rat. But the latter are differentiated from the former by their virulence. Inoculated in the guinea pig, they prove less virulent than the plague cultures; they only kill this animal in three or four weeks, and the lesions observed at autopsy are not characteristic.

Does this fact mean that cultures of rat organic mold are not plague cultures, although their elements are morphologically identical to those of the latter? It does not have this meaning at all; it only means that these cultures, derived from healthy tissues, are less virulent than those derived from rat tissues damaged by a disease of this animal, the plague.

Now, the identity noted above between the Penicilium form of rat organic mold and the Penicilium form of plague mold means that rat plague is an autogenous, spontaneous disease of this animal, resulting from a pathological vegetative transformation of its organic mold.

There is, between the normal organic mold of the rat and plague mold, the same relationship that exists between the normal organic mold of man and the cancer-causing mold or the human tubercular mold.

I have so far outlined the current state of my research. It has been continued by new ones that are currently being carried out and have the following goals:

1. To attempt the reproduction of plague in rats by inoculating bacterial cultures of its specific mold.

2. To increase the virulence of the plague-form cultures already obtained and to verify the lesions they produce with this heightened virulence.

3. To confirm, by isolating and comparing other gonidial forms of the rat's organic mold and the plague mold, the identity already established by comparing the *Penicilium* forms and the bacterial forms.

I have already obtained, in this line of research, the spontaneous transformation of a plague culture on gelatin into a mold of the *Acaulium* type (or *Penicilium brevicaule*) similar to that which was isolated by culturing the organs of a guinea pig inoculated with the substance of a hard, non-caseated tubercle from the tuberculated lung of man. This mold, which I also obtained by direct culture of the caseous matter and tuberculated lung tissue of man, in a very distinct case of tuberculosis, is ochre in color (Fig. 7, 8, pl. 173), and I had related it to the form *Acaulium flavum*. The mold obtained by direct transformation of a plague culture is also ochre in color, like the previous one.

The original plague culture therefore appears to be a coccobacillary bacterial culture of a severe form of spontaneous and contagious rat tuberculosis.

The search for the original source of the plague virus was made difficult by a fact which, in the current state of bacteriological knowledge, would be such as to cause the conclusion just drawn to be rejected a priori as necessarily inaccurate. Indeed, it will be seen, in the study of icterohemorrhagic spirochetosis, that this disease is due, not to a spirochete, but to a pathogenic mold whose original source has been determined: it is the organic mold of the rat.

One would be tempted to conclude from this fact that, if the rat's organic mold causes spirochetosis in humans, it cannot cause the plague. Such a conclusion would be a mistake. Indeed, the human organic mold undergoes the most diverse transformations that correspond to very different diseases: tuberculosis, cancer, pneumonia, numerous diseases characterized by the presence of colibacilli, etc.

On the other hand, inoculating a guinea pig with the various gonidial forms of the same mold causes very different lesions depending on the form inoculated; the *Aspergillus, Penicilium, Monosterigmata,* and *Sporothricum* forms, for example, have different virulence and do not cause the same lesions. The *Monosterigmata* and *Cephalosporium acremonium* forms of the human organic mold tuberculosis guinea pigs, while the *Sporothricum* form of the same mold does not tuberculize them.

The same is true for the rat's organic mold; its various gonidial forms do not have the same virulence and do not cause the same lesions; they cause diseases that have the same original source, but which are clinically different: the *Aspergillus* form causes icterohemorrhagic spirochetosis, while the *Monosterigmata* and *Acaulium* forms cause very different lesions that will be described elsewhere, their study being currently underway.

MALTA FEVER

The pathogen of this disease, *Micrococcus melitensis*, was studied using a culture made available to me by Professor Legroux of the Pasteur Institute and named *M. melitensis 3.* A bacterial culture on agar spontaneously transformed into the *Penicilium* form. Three types of this form are represented in Figures 1, 2, and 3 of Plate 290. These types appeared rudimentary; a more perfect form was sought by inoculating the guinea pig with this *Penicilium* and culturing its organs; the various types thus obtained are contained in Plate 243.

Later, while researching and studying the gonidial forms of organic orange mold, I easily noted the identity of its *Penicilium* form with that of *Micrococcus melitensis* (Plate 244).

From this identification, it resulted that the pathogen of Malta fever is the organic mold of the orange in the form of its bacterial culture. Since there are several races of *Micrococcus melitensis*, I also isolated the gonidial forms of the lemon and sought to obtain bacterial cultures of the orange and lemon by inoculating the pulp of healthy fruits in broth. I thus noted that the *Penicilium* form of the lemon is very similar to that of *M. melitensis* and that the bacterial cultures obtained using lemon and orange pulp are cultures of *Micrococcus melitensis*. The culture obtained with orange pulp is shown in Figures 11, 12, Plate 243.

Studies conducted on cultures of *Micrococcus melitensis* and orange or lemon show that they have a marked tendency to take the *Micrococcus* form, which means that they develop in the form of bacilli that segment very quickly into granules in the cultures; in

fact, rare unsegmented bacilli are found. But this form is not constant; stable bacillary cultures can in fact be obtained; they are sometimes obtained by direct inoculation of fruit pulp or by culturing the organs of guinea pigs inoculated with this pulp.

The pathogenic agent of Malta fever can therefore present in the bacillary form as well as in the *Micrococcus* form.

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The bacilli generally appear as straight, stubby rods, varying in length depending on their stage of segmentation, but generally about 2 to 3 μm long by 0.5 μm wide.

The pathogen of Malta fever is therefore the bacterial culture of the organic mold of the different species of the genus *Citrus* (orange, lemon, mandarin, grapefruit, bitter orange, citron, etc.).

Knowledge of the original source of the pathogen of Malta fever explains why *this disease is localized to the Mediterranean coastal regions, in which the vegetation of species of the genus Citrus (Rutaceae) is also located.* The transport of oranges, mandarins, lemons, etc., in all countries, also explains the isolated cases and small sporadic epidemics of Malta fever for which the source of contagion could not be detected.

In lemon and orange groves, the fruit and leaves rotting in large quantities on the ground are likely one of the most important sources of the virus. The bacterial culture that forms on the soil is carried along by rainwater. It should also be noted that the root vegetation of these plants in the soil develops, as we have seen, an enormous quantity of bacterial culture from these plants, and that this, carried along by rainwater, is also another important source of the virus.

Finally, the fact that cases of Malta fever are observed in regions where plants of the genus *Citrus* do not exist proves that contamination can occur directly through the ingestion of the fruit; it is likely that it is the fermenting fruit, that is, fruit with rotten regions in which the bacterial culture is developed, that determines the contamination.

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CEREBROSPINAL MENINGITIS

I transformed the meningococcus into the *Penicilium* form by inoculating the guinea pig and the rabbit, followed by culturing the pulp of their organs, with a culture designated Meningococcus 1, made available to me by Professor Legroux of the Pasteur Institute.

This *Penicilium* form is represented in the figures on Plate 245. Forms 1 to 5 were obtained by culturing the organs of the inoculated rabbit, forms 6 to 9 by culturing the organs of the guinea pig; they are identical in both cases.

This is the only gonidial form I have obtained so far of this pathogenic mold, which I have not yet identified. I note here its resemblance to certain types of the *Penicilium* form that I isolated from the cerebrospinal fluid of a series of patients suffering from lethargic encephalitis; now, this *Penicilium* form is identical to that obtained by culturing cancerous tissues. This fact has led me to investigate whether meningococcus is one of the bacterial forms of the human organic mold; this research is in progress, as are those of more numerous types of *Penicilium.*

GONOCOCCUS

I was able to obtain a stable culture of gonococcus by inoculating a drop of gonorrhea pus pre-collected from the urethra into glycosylated calf peptone broth; the culture was stored at room temperature. Transferred to agar, it gave rise to pale orange-ochre colonies, the size of lentils.

The culture broth being filtered through a candle, and the apparatus kept intact, small colonies of a mold which was a *Penicilium* (Fig. 7, 8, pl. 247) developed on the external surface of the candle; it was impossible for me to obtain, by successive subcultures, a more developed form than that contained in figures 7 and 8. Figure 6 contains the perfect form of this *Penicilium*, obtained by direct inoculation of gonorrhea pus into a guinea pig and culture of the pulp of its organs. This mold has not yet been identified.

I point out here that the globules of gonorrheal pus, considered as leukocytes, and in which, as we know, enclosed gonococci are found, are germinative masses formed integrally in the culture and have nothing in common with the normal leukocytes of the organism; if gonococci are found in their interior, it is not because they are enclosed there by the phenomenon of phagocytosis, but simply because it is they who, together with the other formed elements of the culture, form these masses by the general phenomenon of agglomeration of the elements of the cultures with a view to multiplication.

LETHARGIC ENCEPHALITIS

I sought to isolate the pathogenic agent of encephalitis lethargica, still unknown, by studying the cerebrospinal fluid of patients in Paris hospitals suffering from this rapidly developing disease.

I also studied the cerebrospinal fluid of two other patients (one from the Cherbourg Civil Hospital, the other from the Besançon Civil Hospital) suffering from a condition

that could only be attributed to encephalitis lethargica, although they did not present all of its characteristic symptoms.

At least two gonidial forms can be obtained from the cerebrospinal fluid of patients by the following methods:

1. By preserving it at room temperature; a mold quite frequently develops there, growing on the surface of the fluid.

2. By inoculating it on agar and various other solid media; a spontaneous mold can develop on any of them.

3. By inoculating it into animals and culturing the pulp of their organs on agar; on the third or fourth day, the cultures become covered with the mold whose elements have been inoculated with the cerebrospinal fluid.

Plate 246 contains a series of types of the *Penicilium* form obtained by culturing the pulp of the organs of guinea pigs injected with cerebrospinal fluid. They relate to two different cases of encephalitis; figures 5, 6, 12, 13 relate to one of them, all the others to the second.

In two other cases of encephalitis, I obtained, for one of them, the *Penicilium* types of Figures 1, 2, Plate 247; and, for the other, the type represented in Figure 4, Plate 247. Figures 3 and 5 of this plate relate to the case of lethargical encephalitis from the Cherbourg hospital. In this case, the *Penicilium* form arose spontaneously and directly from the cerebrospinal fluid **(1)** preserved in a sterile vessel at laboratory temperature.

In two other cases of encephalitis, I obtained a mold presenting in another gonidial form and the same in both cases. The first, Plate 248, was obtained in a case of frank encephalitis in Paris hospitals; the second, plate 249, in a case of encephalitis from the hospital of Besançon.

This mold presents the gonidial form of *Aspergillus minimus* described by Bainier.
In the first case, the gonidial form, although well characterized in figures 4 to 8 of plate 248, is not frank; a large number of fruiting bodies affect another type which is generally found in cultures of the gonidial form *Sterigmatocystis* alongside the gonidial stems characteristic of this form (Fig. 1, 2, 3, plate 248).

It has been established, by exposing the multiplicity of gonidial forms of the same species, that this form of *Aspergillus minimus* (Bainier) is a rudimentary type of the form *Sterigmatocystis* towards which it can evolve by developing further.

(1) *Figures 6, 7, 8, of the same plate relate to the gonococcus.*

In the other case of encephalitis, the gonidial form was clear and presented only the very characteristic *Aspergillus minimus* type (Figs. 1 to 6, pl. 249).

Before attempting to identify the molds just indicated, it is necessary to know whether they are related to the pathogenic agent of the disease, or whether they are simply derived from the organic mold of man, the elements of which are always contained in greater or lesser numbers in the cerebrospinal fluid. It is common for the culture of this normal or abnormal fluid to produce the gonidial forms of the organic mold; but if it also contains the elements of another mold recently introduced into the body by infection, and if it is injected into a guinea pig, it is impossible for the culture of the pulp of the organs of this animal not to give rise to the heterogeneous mold. Experience shows that, on this point, the rule is absolute, without exception.

Now, in ten cases of encephalitis in which the cerebrospinal fluid, *always* cloudy, was tested, I did not obtain any other molds than those I reported. It is therefore certain that the two molds obtained, or at least one of them, relate to the pathogenic agent of the disease. One of these two molds, that of the *Aspergillus minimus* form, is very probably a gonidial form of the organic mold of man; I have in fact obtained an identical mold, presenting this same gonidial form (Fig. 4, 5, pl. 163), *by direct* transformation of a culture of human tuberculosis inoculated on solid media (carrot).

Now, it has been previously demonstrated that human tuberculosis is a vegetative form of the organic mold of man. This gonidial form can obviously belong to other living beings, since Bainier noted its presence on plums, and the scab he found on these fruits was probably a gonidial form of the organic scab of a plum tree. This fact does not remove the value of the previous statement of identity; the value of the objection is diminished, on the other hand, by the fact that the *Penicilium* form obtained from the cerebrospinal fluid of patients suffering from encephalitis is not the *Penicilium* form of the organic mold of the plum tree, but is certainly that of the organic mold of man with which all its types are identified.

This identification can be made by comparing the various types on Plate 46, either with those of human organic mold (Plates 174 and 175), or with the *Penicilium* form obtained by culturing cancerous tissues (Plates 189, 194, 196, 198), which are themselves an abnormal vegetative form of human organic mold. In particular, the comparison of the *Penicilium* types contained in these last four plates with the types on Plate 246 is so suggestive that I did not hesitate to conclude that they are identical.

I must therefore conclude from these facts that *encephalitis lethargica is a spontaneous disease of man, resulting from a pathological transformation of the vegetative form of his normal organic mold. This demonstration results:*

1. On the identification, with human organic mold, of the two gonidial forms *Penicilium* and *Aspergillus minimus*, derived from the elements contained in the cerebrospinal fluid of patients suffering from lethargic encephalitis.

2. On the fact that the injection of this fluid into the guinea pig and the culture of the pulp of the animal's organs did not give rise to other molds, a fact which proves that it did not contain any elements other than those of human organic mold.

Lethargic encephalitis must therefore be included in the framework of autogenous, spontaneous diseases of the human organism; it should have had its place, in this work, following tuberculosis and pneumonia.

This conclusion, verified in ten cases of encephalitis, could only be modified by the subsequent isolation of a heterogeneous mold growing in the nerve centers.

MEASLES

During the microscopic study of the venous blood of a dozen patients with measles, I observed and determined the characteristic elements of the pathogen in the blood and succeeded in isolating and culturing it.

With the first bacterial culture obtained, I was able, by transformation, to obtain the *Penicilium* form, the identity of which, a subsequent study revealed, is that of the organic mold of lettuce called lettuce (*Lactuca sativa*).

Microscopic examination of blood.

The venous blood of the patient in the midst of a feverish and eruptive period is defibrinated or citrated. Placed in a thin layer on a slide, then dried quickly and fixed by heat, it is stained with dahlia; the preparation is then washed in hot water and dried. An irregular spread of the blood is better suited to the examination; it allows one to choose the areas suitable for observation, clear or more opaque areas.

Examination of these preparations shows that the venous blood contains:

1. A large number of large spherical or pear-shaped vesicles, 25 to 50 µm in diameter on average (Fig. 1, plate 250), generally showing traces of the pedicle that carried them; they are generally not very stainable by the dahlia; however, some stain quite brightly; this difference in coloration is due to the fact that some vesicles are empty, others still full. A patient search allows one to find in the preparation the mycelial filaments that give rise to these vesicles. In the center of Figure 1, plate 250, one can see one still adhering to the large mycelial filament that formed it and carrying it late-

rally; in the upper right corner of the same figure, one can see a terminal vesicle carried by a mycelial filament whose segmentation is clearly apparent.

2. Mycelial filaments, generally large, on which the partitioning points can be seen, if they are not too brightly colored.

3. A considerable quantity of spherical bodies whose size varies from 2 to 10 or 12 μ (Fig. 1 and 2, 3, 4, pl. 245). These bodies are the germinative masses of the pathogenic agent; the small or medium-sized ones are in the process of forming by agglutination and agglomeration. Several large masses sometimes come together to form a giant mass. These various germinative masses are visible at a lower magnification, in figure 1 plate 250. These masses are basophilic and to different degrees, depending on the quantity of basophilic elements which are agglomerated there. By careful staining, basophilic masses or nuclei surrounded by lighter protoplasm are perceived in their interior.

4. Bacterial elements. These elements are rare, but nevertheless, it is easy to observe them by scanning a preparation. Several bacilli are visible in Figure 1, Plate 250; one can be seen in Figures 3 and 4, Plate 254; these bacilli are generally about 3 μm long and 0.5 μm wide; they are only one of the bacterial elements in the culture; this is polymorphic; it forms bacilli shorter than the previous ones and of variable width alongside coccobacilli and granules. Only direct examination of these elements in the figures under a magnifying glass can provide exact information on this subject (Figs. 5 to 11, Plate 254). It is likely that at a specific moment in the development, probably at the very beginning, the bacterial elements abound in the blood.

Isolation of the pathogenic agent in the bacterial state.

This isolation is done quite easily by inoculating a matrass of glycosylated peptone veal broth, kept at laboratory temperature (around 18°C), with a few drops of blood taken from a vein of a patient in the midst of a rash.

Transformation of the bacterial culture into a hyphomycete.

The culture can remain in the bacterial state or give rise to submerged mycelial colonies which, over time, end up occupying all the liquid and form a veil on its surface on which aerial fruiting bodies can develop; the first measles culture that I obtained went through these two states (Fig. 2 and 6, pl. 250).

As soon as it was in the mycelial state, microscopic examination revealed the presence, on the mycelium, of vesicles identical to those that had been observed in the same venous blood that had been used for seeding; certain filaments carried a series of these

vesicles (Fig. 6 pl. 250, gr. 100); referring to figure 1, plate 250, it will be seen that the long mycelial filament which it contains must have carried, not just one, but a whole series of similar vesicles, judging by the vestiges of pedicle which it presents on its path.

In other regions, terminal vesicles existed which were perfectly identical to the one which is reported in the upper right corner of figure 1 plate 250.

In another case, the venous blood of the patient, inoculated directly onto solid media, gave rise on cooked beef to a *Penicilium* form bearing fruiting bodies, at first very rudimentary (Figs. 3, 4, 5, pl. 250), then more developed although still imperfect (Figs. 7, 8, pl. 250).

Microscopic examination of the rudimentary type of fruiting bodies revealed a host of terminal vesicles identical to those found in the venous blood of patients (Figs. 3, 4, 5, pl. 250); this examination further showed that the vesicles were the sacs in which the fruiting bodies of the *Penicilium* form of the pathogen are formed.

The gonidial forms of the measles pathogen can be obtained by the following processes:

In blood cultures from patients on agar, a thick, cloudy, or opaque material forms, which flows to the bottom of the tubes and is a bacterial culture of the pathogenic agent. Transferred to various solid media, this bacterial culture gives rise directly to various gonidial forms of the pathogenic mold; they are also obtained, and much more quickly, particularly the *Penicilium* form, by inoculating this culture into the guinea pig and culturing the pulp of its organs on agar.

They are also obtained by directly injecting two or three drops of venous blood from a patient into the guinea pig and culturing the pulp of its organs. But, in this case, careful control of the molds obtained must be carried out, as the gonidial forms of the pathogenic mold can be accompanied, in the cultures, not only by those of the guinea pig's organic mold, but also by those of the human organic mold.

Plate 251 contains the types of the *Penicilium* form obtained from the venous blood of three patients (measles No. 5, Figs. 1, 2, 3; measles No. 9, Figs. 4, 5; measles No. 10, Figs. 6, 7, 8, 9). Later, I obtained these *Penicilium* with more perfect types, identical to those in plate 252, by successive subcultures. Plate 252 contains, in addition to two types from measles cases no. 5 (Fig. 10) and no. 10 (Fig. 6), the most characteristic types

of the *Penicilium* form obtained in another case (measles no. 3). The culture of *Penicilium* corresponding to this case was chosen for this exhibition because it derives from a culture that was first bacterial, then mycelial, and presented the characteristic elements observed in the blood of patients. Its origin is therefore certain.

Identification of the Penicilium form of the pathogen.

The types of the *Penicilium* form of the measles pathogen (Figs. 1 to 10, pl. 252) are very distinct from those of the organic molds of guinea pigs and humans. In its perfect form, this *Penicilium* has a mycelium with large filaments ending in fruiting groups of a distinct shape which allow it to be recognized as the *Penicilium* form of the organic mold of salad called lettuce (*Lactuca sativa*). This identification requires a rather lengthy examination because of the polymorphism of this species when it is obtained by direct culture of the pulp of the lettuce stem. Identification is more easily achieved by the direct study of preparations of the mold than by comparison of photographs, because many fruiting bodies suitable for direct examination do not lend themselves to photography. Nevertheless, the plates provide convincing elements of comparison relating either to the shape of the terminal fruiting bouquet (Fig. 7, pl. 253 (lettuce), fig. 8, 9, pl. 252 (measles)), or to the shape of the groups of sterigmata (Fig. 6, pl. 252 (measles), fig. 7, pl. 253 (lettuce)), or of the sterigmata in isolation (fig. 7, pl. 253 (lettuce), fig. 6, pl. 251, (measles)) or to the shape of the abnormal sterigmata (Fig. 1, pl. 253 (lettuce), fig. 8, pl. 250, (measles)), or to the shape of the lateral branches (Fig. 5, pl. 253 (lettuce), fig. 2, pl. 4, 250, (measles)..., etc.

Identification of bacterial cultures of the measles pathogen.

The identification just made is confirmed by comparing the elements of the bacterial cultures of the lettuce organic mold with those of the cultures of the pathogen isolated from the blood and with those contained in the blood itself. This comparison will be easily made by examining plates 254 (measles) and 255 (lettuce).

We have seen that the blood of patients with measles contains a large quantity of spherical masses of varying size, basophils (Fig. 1, 2, plate 254) very distinct from blood cells and leukocytes. These are the germinative masses of the bacterial culture of the pathogenic agent.

It is easy to obtain a bacterial culture of the organic mold of lettuce; it is sufficient to inoculate a little aseptic pulp from the central part of the stem of the plant into broth, preserved at 18-22°C. In such a culture, the formation of the bacillary elements repre-

sented in Figures 5, 6, Plate 255 is observed on the first day; then, on the second and subsequent days, the formation of very numerous germinative masses, teeming in the broth (Figs. 1, 2, 3, 4, Plate 255), identical to those observed in the blood of patients is observed.

This element is most important for identification, due to the considerable quantity observed in the blood of patients.

The first culture I obtained, by inoculating blood from a patient with measles into broth, produced a bacterial culture in about eight days, then a surface film whose elements are represented in Figures 7 to 11, Plate 254. A small fragment of this film was placed in a drop of water on a glass plate, dissociated, spread, dried, and fixed by heat, then colored with dahlia; Figures 7 to 11 represent the elements contained in different areas of such a preparation; in Figure 10, we see the large and small mycelial elements constituting the film, the latter in the process of bacillary segmentation; Figures 7, 8, 9, and 11 show the shape of the bacterial elements released by segmentation.

We see from these figures that the segmentation or bacterial elements are very polymorphic. We find in the cultures:

1. A large, basophilic bacillary form, 4 to 6 µm long and approximately 0.6 µm wide, which segments into shorter bacilli. This form is contained in Figures 6 (measles 10) and 10 (measles 3), Plate 2543. These bacilli result from the segmentation of long mycelial filaments from much larger branches that formed the framework of the culture veil.

This bacillary form does indeed belong to the pathogenic agent of measles, since the mycelial branches of the measles culture 3 bore lateral or terminal vesicles identical to

those contained in the blood of patients (Fig. 6, pl. 250), a fact which attests to its origin and which has already been explained above.

2. In the first days, the culture contains a bacillary element that is shorter and less thick than the previous one (2 to 3 µm long by 0 µm. 3 to 0 µm. 4 wide); Figure 4, Pl. 254, represents this element observed in the same culture (measles 5) where the previous large form (Fig. 6) appeared from the sixth to the seventh day.

3. Large and medium-sized bacillary elements, but which no longer take on basic colors with difficulty because they have lost their basophilic protoplasm; they now contain only a small mass constituting one or two polar globules in the bacillary element (Fig. 8, Pl. 254). A few rare bacilli of this form are found, with large and rare basophilic bacilli, in the blood of patients during the eruption (Fig. 3 and 4, Pl. 254, measles 5).

4. Short, extremely numerous elements in the form of coccobacilli, often possessing one or two polar globules. These coccobacilli come from the segmentation of the preceding bacillary forms, or, directly, from the segmentation of the mycelial filaments which give rise to them (Fig. 8, 9, pl. 254, measles 3). These coccobacilli are found in the blood of patients with eruption, but are rare.

Let us now compare these various elements with those of a bacterial culture of organic lettuce mold. We have seen previously that such a culture contains, from the second day, a large quantity of germinative masses (Fig. 2, pl. 255) identical to those formed by the pathogenic agent in the blood of patients. They are found in both old and young cultures. They are often gathered in a large mass (Fig. 2); they emit mycelial filaments which form bacilli by segmentation (Figs. 1 and 2); by carefully examining the germinative masses of the blood, these filaments are also observed.

I must mention here that these germinative masses are, in vitro, the leukocytes of the culture and that they present an absolute morphological identity with the leukocytes of animals, which can easily be convinced of by examining Figure 2, Plate 255; there is a new example demonstrating the existence of leukocytes both in bacterial cultures and plant tissues as well as in animals. In Figures 3 and 4 of the same plate, we find germinative masses at different stages of formation by agglomeration; this is also a further example of the origin and mode of formation of these elements.

Bacterial cultures of lettuce, one to two days old, contain a large mass of bacilli (Figs. 5 and 6, pl. 255) identical to the average bacillary form of the measles pathogen (compare Fig. 5, pl. 254). They contain small and irregular bacillary elements that are also observed in measles bacterial cultures (Fig. 7, pl. 254). Figures 2, 8, 9, plate 255, clearly demonstrate the origin of these bacilli.

From the second day onwards, the mycelial filaments segment, either into bacilli 3 to 4 µm long, or into coccobacilli (Fig. 4, 7, 8, 9, pl. 255, (lettuce)) identical to those observed in old bacterial cultures of measles (Fig. 9, pl. 254 (measles)).

Certain mycelial filaments, whose elements have not yet dissociated, clearly show segmentation into long bacilli and their subsequent division into coccobacilli (Fig. 8, pl. 255).

In addition, in old cultures, we find bacillary elements that have lost their basophilic protoplasm, of which they now contain only a small quantity in the form of polar globules (Figs. 1 and 3, pl. 255). Identical elements, which have been described above, exist in the blood of patients and in bacterial cultures of measles (Figs. 8 and 11, pl. 254).

Thus, in summary, we observe the same elements in a bacterial culture of the organic mold of lettuce as in a bacterial culture of the measles agent.

It may be surprising that I did not observe in abundant quantities, in the blood of patients, the bacterial forms that I obtained in in vitro culture. This is a common and well-known fact in the study of diseases. A pathogenic germ, known and well-identified, appears not to vegetate in the blood, because, when we look for it, it is not there. It has often been concluded from this fact that the pathogenic element does not vegetate in the blood. This is obviously an erroneous conclusion. If the sought-after bacterial element is not found in the blood, it is not because it is not there, but because it is no longer there. Bacterial cultures often evolve more quickly or *more completely* in the animal organism than in vitro; *the end of bacterial evolution is characterized by the passage of the elements to the state of large germinative masses and to the stable mycelial state.*

In this state, the bacterial elements, especially the bacillary elements, become considerably rarer, and the evolution of the pathogenic mold can take place, from this moment, by granulations (spores) which become difficult to distinguish from normal granulations belonging to the infected organism.

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For measles and scarlet fever, and I think all eruptive fevers, the case is very clear; only rare bacillary elements are found in the patient's blood; but, on the other hand, a considerable mass of germinative masses is observed. *The reason for this is that the eruption, which is the essential element of the diagnosis,* is caused by the cessation of the germinative masses and their vegetation at the level of the cutaneous blood capillaries, where they cause the phenomena that constitute the eruption (or in internal organs where they are the cause of a congestive lesion). Now, I only had at my disposal blood from patients with an eruption, whose *bacterial development is almost complete.* To observe bacterial pulmonary infestation in measles or scarlet fever, it would therefore be necessary to look for it in the blood *at the beginning or during the feverish period preceding the eruption.*

To summarize, we have observed a double identity in the gonidial form of *Penicilium* and in the bacterial forms between the measles pathogen and the organic mold of lettuce. *I conclude that this mold is the pathogen of measles and lettuce the original source of the virus of this disease.* Research is continuing to complete this identification of the pathogen by isolating its other gonidial forms.

This explains why the measles virus is so widespread that very few children escape the disease; the soiling of the mother's hands by handling lettuce, a daily food, and the contamination of the child's hands and face by the mother's hands, explain the almost

fatal contamination of children. Lettuce produces a bacterial culture with the greatest ease by rotting in a household garbage container; at the same time as this culture, a mold, *Bremia Lactucae*, forms, whose spores can be an agent of contamination. To obtain this mold, simply place lettuce leaves in a small vase (glass or pot) covered with a plate; after two to three days, the leaves are completely covered with a gray mold that could be mistaken for *Botrytis cinerea*, but which, in reality, is *Bremia lactucae.*

It is also possible that contamination results from the ingestion of the salad.

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SCARLET FEVER

The specific agent of scarlet fever is generally considered unknown, the specificity of the streptococcus observed in the blood and throats of scarlet fever patients being problematic.

I searched for the agent of this disease, either by microscopic examination of venous blood taken aseptically from a vein of a dozen patients in the midst of an eruption, or by culturing this blood in broth and on various solid media. This examination demonstrated that the pathogenic agent of scarlet fever is a mold whose presence can be detected in the blood of patients by its mycelial vegetation and fruiting bodies. I was able to obtain the bacterial culture and several gonidial forms of this mold, then achieve its identification, which provides knowledge of the source of the disease virus: it is the organic mold of carrots.

Elements of the pathogenic mold in the blood of patients.

These elements can be searched for either in citrated blood or in coagulated blood. Citrated blood is preferable. It is important to know, in fact, otherwise the search would be fruitless, that mold exists in the blood in the form of long mycelial filaments, often grouped in balls (Fig. 2, pl. 256), which are enclosed by the clot; they would therefore be searched for in vain in the serum, which contains only isolated fruiting bodies of the mold (Fig. 5) or bacterial elements.

But we can observe this mycelial vegetation in the clot if we dissociate it finely or if we crush a small piece between two slides that we slide one over the other; we fix it by drying quickly on a hot plate and we stain it hot with dahlia. The mycelial vegetation of the pathogenic mold appears in the form of long flexuous filaments, gathered in more or less large bundles (Fig. 2, pl. 256), or sometimes in groups of only four or five (Fig. 4, pl. 256) and ending in a fructification in the form of a spherical ball; they bear laterally, either ramifications ending in a spherical ball identical to the previous one, or isolated, short sterigmata, ending in a spore; a careful examination allows us to see that some

emit a string of spores; we distinguish these sterigmata on the mycelial filaments by careful examination, under a magnifying glass, of figures 2 and 4.

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There are also larger mycelial branches in the blood; some of them bear fruiting bodies in the form of spherical or slightly pyriform vesicles of 40 to 45 µm on average, carried by a pedicle of length approximately equal to that of the fruiting body (the vesicles of measles are sessile or subsessile). A patient search allows one to observe mycelial branches still bearing a series of pedunculated vesicles which they have formed (Fig. 6, pl. 256).

The blood contains a large quantity of these free vesicles; they are also 40 to 45 µm in diameter. Some appear empty, others are stuffed with round spores of 2 to 3 µ 5 (Fig. 5); on most, a conical projection indicates the point of attachment of the pedicle, a part of which often remains adherent to the vesicle; the blood also contains a large number of isolated spores of 2 to 3 µ 5 or smaller.

Isolation of the pathogenic blood mold and search for its gonidial forms

One of the forms of the pathogenic mold can be obtained by the following means:

1. By spreading scarlet fever scales on solid media (agar, carrot), the external face of the epidermis being applied to the culture medium.

2. By spreading blood or the crushed clot on solid media (agar, carrot, etc.).

3. By inoculating the blood in broth and transferring the bacterial culture, or that which can be obtained by other methods, onto solid media.

4. By culturing the pulp of the organs of an animal inoculated with the bacterial culture of the pathogenic agent, or with the scales crushed in water, or even with a drop of venous blood from the patient; in the latter case, especially, it is necessary to examine the molds obtained in order to avoid confusion with the organic molds of humans and guinea pigs.

By these different methods, I obtained the following gonidial forms:

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1. Monosterigmata Form

By cultivating blood on carrots, I obtained a short, white mold, consisting of long creeping filaments bearing, as well as their lateral branches, terminal ball-shaped fruiting bodies and, in addition, laterally, either slender branches ending in balls, or short or well-developed sterigmata, ending in a spore or a string of spores (Fig. 1, 3, pl. 256).

This mold is not the typical or perfect monosterigmata form; it is only a rudimentary form. I obtained the perfect form either by culturing the pulp of animal organs inoculated with a scarlet fever bacterial culture, or directly by culturing the scales. This form is identical to that shown in Figure 4, Plate 278 (rabies), which is also the monosterigmata form of the same pathogen, organic carrot mold.

This monosterigmata form itself evolves and forms gonidiophores bearing three to five sterigmata.

The rudimentary monosterigmata form evolves, in vitro, into the perfect form, because, on filaments of the rudimentary form still bearing terminal balls, we find perfect sterigmata emitting large spores in a string.

The monosterigmat form is certainly the form, or at least one of the forms, that the pathogenic mold takes in the blood of patients; indeed, by examining Figure 2 containing a bundle of mycelial blood filaments, we will see that these emit short lateral branches bearing balls at their ends and sterigmata ending in a spore. These filaments therefore relate to the monosterigmat form.

But it is probable that the pathogenic mold also vegetates in the blood in other forms that are very common to it, for example in the *Corymbisporium* form (Fig. 18, pl. 260 (carrot), and Fig. 5, pl. 278 (rabies)), and the *Acremonium alternatum* form.

2. Form Acremonium alternatum or Cephalosporium acremonium.

Another form, *Monosterigmata*, is also obtained by culturing scarlet fever scales or the pulp of guinea pig organs inoculated with blood, or scales ground in water, or a scarlet fever bacterial culture.

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This gonidial form presents, on very long mycelium filaments, long and thin gonidiophores, ending first in a ball; when this is detached, they emit oval, terminal spores, which sometimes remain grouped at the end of the gonidiophore. This gonidial form is therefore identical, depending on its state, to the two forms designated: *Cephalosporium acremonium* and *Acremonium alternatum*; there is identity between these two forms. When the spores grouped at the head of the gonidiophore are detached, the latter emits spores which detach one by one, or a string of spores; it therefore loses the *Cephalosporium* type to take on the *Acremonium* type.

3. Verticilium form.

It developed spontaneously on the venous blood of a patient preserved in a bottle where it formed, on the surface of the liquid, small white colonies, then a continuous

veil, consisting of long creeping filaments bearing fruiting bodies represented in Figs. 1 and 2, plate 257 (scarlet fever 8). Among the mycelial filaments, we observe some which bear only isolated sterigmata and are similar to those contained in Fig. 1, plate 256. This mold is very similar to a gonidian form of organic mold of the potato (Fig. 5, plate 146) which I also obtained by transformation of the tetanus mold identified with organic mold of the potato.

Does this gonidial form belong to the potato, the agent of smallpox and cowpox, or to the carrot, the agent of scarlet fever? This question was resolved by the spontaneous transformation of the mold into a *Penicilium* form, a number of types of which are represented in Figures 1, 10, 11, 13, 15, and 16. This form did not present any branched type that could allow it to be identified with the *Penicilium* form of the potato. On the other hand, certain simple types (Figs. 1, 10, 11, 13, 15, and 16, pl. 258) were identified with the *Penicilium* form of the carrot (Figs. 1, 5, 11, pl. 259; Figs. 5, 7, pl. 260). This gonidial form must therefore be attributed to the organic mold of the carrot.

4. Form Aspergillus minimus (Fig. 3, pl. 257).

I obtained it by culturing the scales on agar. It is a mold that develops in the form of rounded colonies with a yellowish edge and an olive-green or pale yellowish-green center. The necessary steps to identify this conidial form have not yet been completed.

5. Citromyces form (Fig. 6, pl. 257, scarlet fever 3)

Obtained by culturing scales on agar. It is clearly distinct from the human form; some filaments in the culture evolve into the *Penicilium* type (on the left of the figure).

6. Penicilium form.

I isolated it directly, either by culturing scales on agar (Fig. 5, pl. 257, scarlet fever 7), or by spontaneous transformation of other gonidial forms obtained directly. I obtained it indirectly by culturing the organs of guinea pigs and rabbits inoculated either with a scarlet fever bacterial culture, with scales crushed in water, or with a drop of venous blood from the patient (Fig. 4, pl. 257, scarlet fever 6. Inoculation of blood in the rabbit).

The *Penicilium* form should not be judged by the rudimentary types represented in forms 4, 5, and 6, Plate 257, which are those of the first culture obtained; by several successive subcultures on suitable media, these types have taken the perfect form represented by the figures on Plate 258. It is indeed often necessary to subculture a culture on sugar agar several times to bring it to its perfect state of development.

Plate 258 contains various types borrowed from the *Penicilium* culture of four different cases of scarlet fever (scarlet fever 1, 3, 5, and 8).

The types in cases 1 (Figs. 2, 3, 4, 5, 9, 12, 17) and 3 (Figs. 6, 7, 8) were obtained indirectly, the former by inoculation of the bacterial culture into the guinea pig, the latter by inoculation of venous blood into the rabbit. The types in cases 5 (Fig. 14) and 8 (Figs. 1, 10, 11, 13, 15, 16) came from direct production; the former from a culture of scales on agar; the latter from a direct transformation on agar of the *Verticilium* form (Figs. 1 and 2, pl. 257) developed in the venous blood itself.

7. Aspergillus form.

I isolated it by culture of the scales and by culture of the pulp of the tissues of the guinea pig inoculated with blood from a patient suffering from scarlet fever.

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Identification of the pathogenic mold of scarlet fever.

The identification of the pathogenic agent of scarlet fever cannot be separated from that of the pathogenic agent of rabies. I identified the agent of scarlet fever, first, by comparing its *Penicilium* form with that of carrot. I then sought to obtain the group of gonidial forms of the organic mold of carrot, to compare them with those of scarlet fever; I thus successively obtained four gonidial forms from carrot, whose identity I immediately perceived with four gonidial forms of the agent of rabies; the identity of the *Penicilium* form of carrot with that of rabies was then established.

These findings required a new search for identity between the gonidial forms of the agent of rabies and those of the agent of scarlet fever. This check was demonstrative; the identity was complete. Let us first recall which gonidial forms were obtained for each of the molds discussed here.

For the organic carrot mold, the following were obtained:

An unclassified inferior form (Figs. 15, 16, 17, pl. 260).

The rudimentary *Monosterigmata* form (not shown).

The perfect *Monosterigmata* form (not shown).

The *Acremonium alternatum* and *Cephalosporium acremonium* form (not shown).

The *Corymbisporium* form (Fig. 18, pl. 260).

The *Mucor* form (not shown).

The *Aspergillus* form (not shown).

The *Penicilium* form (Pl. 259-260).

For the scarlet fever pathogen, the following were obtained:

The rudimentary *Monosterigmata* form (Fig. 1, 3, pl. 256).

The perfect *Monosterigmata* form (not shown).

The *Acremonium alternatum* and *Cephalosporium acremonium* form (not shown).

A special *Verticilium form* (Fig. 1, 2, pl. 257).

The *Citromyces* form (Fig. 6, pl. 257).

The *Penicilium* form (Pl. 257, 258).

The A*spergillus* form (not shown).

The *Aspergillus minimus* form (Fig. 3, pl. 25…**?**)

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For the rabies pathogen, the following were obtained (Pl. 278):

The perfect *Monosterigmata* form (Fig. 4, pl. 278).

The *Cephalosporium acremonium* form (Fig. 3).

The *Acremonium alternatum* form.

The *Verticilium* form, of which the previous one is a reduced form.

The *Corymbisporium* form (Fig. 5).

The *Aspergillus* form (Fig. 1).

The *Mucor* form (Fig. 2, pl. 278; fig. 1, pl. 279).

The *Penicilium* form (Figs. 6 to 8, pl. 278; figs. 2 to 16, pl. 279).

All forms not shown in the plates have been photographed and combined into new supplementary plates. These, established too late, have not been inserted into this work, due to the need not to delay its publication; they will therefore be published later elsewhere.

Moreover, most of these gonidian forms exist in one of the plates indicated. The identification of the *Penicilium* form of the agent of scarlet fever and rabies with that of the organic mold of carrots was difficult at first, because the latter is quite polymorphic. I had thought that it was safer, for this identification, to take as a term of comparison the types of the *Penicilium* form developed directly, that is to say on the very tissues of the carrot. This is an error; these types must be used, but it is necessary to establish the comparison with the types of the *Penicilium* form obtained by passage of the organic mold of carrots in the organism of the guinea pig; animals are inoculated with carrot juice and the pulp of their organs is cultured on agar after death; the *Penicilium* form

always develops on these cultures. In addition, a creamy, whitish material develops there, which spreads around the seeded fragments and which is made up of a bacterial culture of the organic mold of the carrot; this culture, as well as the one it reproduces in broth, are used for identification with the cultures of the pathogenic agent of scarlet fever and rabies.

With the help of these materials, the identification of the *Penicilium* forms becomes easy.

It is necessary to point out here that the plates before the reader were not made deliberately to prove the identification; they were made to establish it, before I knew it;

none of the types shown was therefore specially chosen; the types photographed are only those which appeared the most characteristic.

From the comparison of the gonidial forms of the organic mold of the carrot with those of the pathogenic agents of scarlet fever and rabies, it results:

1. That the rudimentary *Monosterigmata* form of the carrot is identical to that of the agent of scarlet fever and that the perfect form is identical to that of the agents of scarlet fever and rabies.

2. That the *Acremonium alternatum* and *Cephalosporium acremonium* form of the carrot is identical to those of the agents of scarlet fever and rabies.

3. That the *Corymbisporium* form of the carrot is identical to that of the agent of rabies.

4. That the *Aspergillus* form of the carrot is identical to that of the agents of scarlet fever and rabies.

5. That the *Penicilium* form of the carrot is identical to that of the agents of scarlet fever and rabies.

6. That the gonidial forms of the pathogenic agents of rabies and scarlet fever, when directly compared, are identical.

7. That the rudimentary *Mucor* form of the rabies agent develops a large quantity of pedunculated chlamydospores (Fig. 1, pl. 279), identical to those that the pathogenic mold of scarlet fever develops in the blood of patients (Figs. 5 and 6, pl. 256).

The fruiting filaments of the *Penicilium* form of carrots occur in two forms: a form with large, stubby filaments (Pl. 259, and figs. 1 to 3, pl. 260) and a form with slender filaments (Figs. 4 to 14, pl. 260).

The *Penicilium* form of the scarlet fever agent most often occurs in the large form, while the *Penicilium* form of the rabies agent occurs indistinctly in the large (Figs. 6 to 9, pl. 278) or slender (Figs. 2 to 16, pl. 279) forms.

Comparing these last two large forms, one would be tempted to conclude that they belong to two different species. But, by studying the various types of the *Penicilium* form of carrots, one sees that they also include both the large and slender forms.

The slender form of the rabies agent (Figs. 2 to 16, pl. 279) relates to the various types (5 to 14, pl. 260) of the carrot; the large and squat forms (Figs. 6 to 9, pl. 278) relate to types 1 to 3 of plate 260 (carrot), to those of Plate 259 (carrot), and to those of Plate 258 (scarlet fever).

Complete identity is thus obtained for five gonidial forms between the organic mold of the carrot and that of the agents of rabies and scarlet fever; four of these gonidial forms are common to the agents of rabies and scarlet fever.

We have also seen previously that the septic vibrio is identified with the bacterial form of the *Corymbisporium* type of the carrot, which is its original source.

We will see, in the study of rabies, that in the organs of rabid animals, we find a bacterium which is the septic vibrio.

Let us now investigate whether the study of bacterial cultures of carrot, scarlet fever, and rabies confirms the identification which has just been established.

Identification of bacterial cultures of scarlet fever and rabies with those of organic carrot mold.

A bacterial culture of the scarlet fever agent is easily obtained, either by inoculating scales in broth or by culturing the patient's blood on agar; in the latter case, rounded colonies formed by a white or yellowish creamy material, which is the bacterial culture, develop on the surface of the agar.

To establish a comparison between the bacterial forms of these cultures and those of the organic mold of carrots, it is necessary to obtain the latter by two processes: directly, by inoculating in broth a little juice taken by scraping the center of a carrot broken in two, and indirectly, by culturing on agar the pulp of the organs of a guinea pig inoculated with a portion of the same juice; a whitish material develops on the agar, which gradually spreads and ends up sinking to the bottom of the tubes; this material, consisting of the bacterial culture, is inoculated in broth. In comparisons, the bacterial forms on agar and in broth must be taken into account.

The forms must be compared, of course, especially for broth cultures, taking into account their age, as the culture evolves as it ages.

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In plate 261 the bacterial forms in parallel various cultures of two cultures of the scarlet fever agent on the sixth and thirteenth days, on the left (Fig. 1 to 3, scarlet fever 6 and fig. 5, 7, scarlet fever 2), and those of various cultures of organic carrot mold aged six to seven days (on the right, fig. 2, 4, 6, 8, 9). Here, in both cases, it is a question of cultures in peptone, glycosed calf broth, preserved at room temperature (23-24º at the time of these cultures).

Without the need for a detailed description, the reader will note an identical polymorphism of the elements in both cases, and the identity of the bacterial forms. In bacterial cultures of carrot and scarlet fever aged two to six days, bacilli of various lengths and widths are found, among which is a large bacillus 5 to 8 µm wide, by 2 to 2 1/2 µm long (sometimes longer). I have found this large bacillary form in the blood, in the pericardial fluid, and in the cerebral substance of a dog suffering from rabies; this bacillary form has been represented in Figure 2, Plate 19, and drawn at a time when I was unaware that it belonged to the specific agent of rabies.

In a thirteen-day-old scarlet fever culture, I observed (Fig. 7, scarlet fever 2) numerous large bacillary elements that had lost their basophilic protoplasm, only a portion of which remained in the form of one or two polar globules, or several granulations.

While searching in bacterial cultures of carrots that were six to twelve days old, I found these same forms; to observe them easily, it is necessary to stain the preparation sparingly with dahlia.

They are also observed almost constantly in cultures of the rabies agent on agar, which affect *the bacillary form;* the bacilli, as they age, gradually lose their basophilic protoplasm. At a given moment, they contain only three or four, then two or just one, and eventually become barely stainable with basic colors.

Six- to twelve-day-old carrot bacterial cultures also contain another element shown in Figures 8 and 9 of Plate 261 (Six-Day Broth Cultures). These are chains of more or less long granulations. At the beginning of their formation, they are first continuous mycelial filaments, then chains of bacilli, and then coccobacilli when the segmentation points are marked; subsequently, each short coccobacillus divides into two granulations; a chain of streptococci is thus formed.

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Examining these chains, particularly the shorter ones, formed of four to six elements, I found them identical to the short chains (quite rare) that I observed in the venous

blood of patients, and which have been reported by many observers. The organic mold of the carrot therefore gives rise, alongside the other bacterial forms, to chains of coccobacilli and chains of streptococci.

The presence of streptococci in the throat or blood of scarlet fever patients is therefore explained; the presence of these chains being quite rare or uncommon in in vitro carrot cultures, it is also explained that observation does not often detect them in the blood of patients. It is, in fact, bacillary bacterial forms that constitute the almost unique elements of young cultures; the bacillary and coccobacillary forms are generally only shown in cultures five to six days old or more.

We should therefore observe numerous bacillary elements in the blood of patients. If their presence has not been noted, it is certainly because they only exist in the blood at the beginning of the disease, at the time of the appearance of angina and before the eruption. The eruption is probably determined by the arrest, in the capillaries, of the coils of mycelial filaments of the pathogenic mold and the groups of germinative masses which give rise to them. This mycelial vegetation does indeed exist in the blood of patients with eruption (this is demonstrated above), the period of bacillary vegetation is *therefore prior to the eruption.* It is therefore probable that a very early examination of the blood of scarlet fever patients will reveal the existence of bacillary forms.

Proof of the arrest of the mycelial elements of the mold and their vegetation in the capillaries is given by microscopic examination of the inner surface of the scales, slightly colored, either by transparency under strong lighting or after dissociation. The presence of a considerable quantity of fruiting bodies and mycelial elements of the mold is observed. It is therefore the vegetation of the latter which, by the mass of the elements formed, raises the epidermis and causes desquamation.

The facts presented demonstrate the identity between the bacterial cultures of the pathogenic agents of scarlet fever and rabies and those of the organic mold of carrots; they therefore confirm the identification already established by the study and comparison of the gonidial forms.

We must therefore conclude:

1. *That the pathogen of scarlet fever is the organic mold of the carrot.*

2. *That the pathogen of rabies is also the organic mold of the carrot.*

3. *That, consequently, rabies is not a distinct disease, and that it is a cerebral form of scarlet fever in dogs or, more precisely, of the injection of the dog by the organic mold of the carrot.*

The bacterial culture of the carrot can develop by the prolonged stay of particles of this food in the mouth or on the tonsils, the latter appearing to be, for the angina that is constant in the disease, the entry point of the virus. The cooked carrot, whose zymospores are not destroyed, can develop a bacterial culture; indeed, the cooked carrot becomes sour (fermentation) after a few days, as a result of the bacterial vegetation of its organic mold not completely destroyed by cooking.

Carrot eaten raw can develop its bacterial culture much more easily than cooked carrots. A housewife can contaminate those around her or herself with hands soiled with the juice of a freshly peeled carrot. The pulp removed by scraping, spread on tables or the floor, is another source of contamination.

RABIES

I was able to carry out research on the pathogenic agent of rabies using the following materials:

1. A dog with rabies, made available to me by Mr. Vasseur, a veterinarian at the pound, as well as the spinal bulbs, preserved in glycerin, of two other rabid dogs that he had autopsied eighteen months and two years previously.

2. Fresh marrow from rabid rabbits made available to me by the Pasteur Institute.

The brain matter from the Ammonian horn of the first dog was reduced to a pulp and cultured partly on agar and partly in broth.

Three different molds developed on the agar cultures, exhibiting the following fruiting forms:

1. A gonidial form which was later identified with the forms *Cephalosporium acremonium* and *Acremonium alternatum* (Fig. 3, pl. 278).

2. A gonidial form *Corymbisporium*.

3. A *Penicilium* form.

A bacterial culture was obtained; it formed a veil which developed into a long-haired mold, a gonidial form intermediate between the *Mucor* form and the *Aspergillus* form (Fig. 2, pl. 278). The bacterial culture was inoculated into a rabbit's hind leg; the enlarged groin lymph nodes were reduced to a pulp and cultured on agar; a mold of the gonidial form *Monosterigmata* developed on the cultures (Fig. 4, pl. 278).

Later, from two rabid rabbit marrows from the Pasteur Institute, reduced to pulp and cultured on agar, I obtained a *Penicilium* form (Fig. 6 to 9, pl. 278) identical to that

already obtained previously, an *Aspergillus* form (Fig. 1, pl. 278), the *Acremonium alternatum* form, and the *Verticilium* form.

Later, I inoculated a dog, intracerebrally, with an emulsion of rabid dog bulb made available to me by Mr. Vasseur; the animal was struck with characteristic rabies and succumbed within fifteen days after exhibiting the following symptoms: intermittent high-pitched cries, hallucinations, sadness, dejection, fits of furious barking with temporary excitement, then paralysis and death; the animal presented at autopsy a severe cerebral congestion, congestion of the larynx, of the trachea and subcutaneous cellular tissue of all regions, pulmonary congestion, congestion of the liver and spleen.

The brain of the animal was removed and preserved in glycerin; with the pulp of the substance of Ammon's horn, a series of cultures on agar were made; a *Penicilium* form and an *Aspergillus* form identical to the previous one developed there; on the section of Ammon's horn of the brain preserved in glycerin and which emerged from the liquid, the same *Aspergillus* form developed abundantly and directly as that of the cultures.

The spores of this *Aspergillus* form were inoculated intracerebrally into a dog, a rabbit, and two guinea pigs. After three days, the dog suffered a fit of furious rage that lasted about an hour and a half and ended in the animal's death from paralysis. The attack was characteristic, for the animal, howling and salivating profusely, continued to bite the wooden sleepers of the cage floor, lacerating them.

At autopsy, it presented the same characteristic lesions as the animal from which the inoculated culture had come: violent cerebral congestion, severe congestion of the larynx, trachea, and subcutaneous cellular tissue, congestion of the lung, liver, and spleen.

A large bacterium, identical to the septic vibrio, 1 to 1 μ 2 wide and of variable length, was swarming in the organs: brain, liver, spleen, etc. This bacterium was also swarming in the organs of the previous dog, and I had already noted its presence in the organs of another dog suffering from street rabies made available to me by Mr. Vasseur.

The rabbit suffered paralysis on the fourteenth day after inoculation and died the next day.

The two guinea pigs died, one after sixteen days, the other after twenty-three days; the first had suffered paralysis of all four limbs twenty-four hours before death; no paralysis was observed in the second, which died during the night.

A new rabbit received, via the intraspinal route, an emulsion of bone marrow from the previous dog that had died of rabies after intraspinal inoculation with cultured *Asper-*

gillus spores; it suffered paralysis of all four limbs the next day and died on the fifth day.

Other tests, carried out later with the same culture on dogs, gave irregular results: sometimes a characteristic paralytic rabies causing death in about ten to thirty days, sometimes temporary phenomena of paresis, followed by recovery; sometimes, but rarely, the animal showed no symptoms. I think that, in the latter case, the culture did not develop.

The inoculated *Aspergillus* form therefore caused rabies, without any possible doubt. Being in possession of this form of the rabies pathogen, I sought to transform it into other gonidial types, in order to verify whether they corresponded to those I had already obtained, either from another rabid dog, or by culturing the marrow of rabid rabbits from the Pasteur Institute.

This transformation was sought by inoculating the guinea pig and the rabbit with the spores of the *Aspergillus* form and culturing the pulp of their organs on agar. I thus obtained:

1. A *Penicilium* form occurring in two types, either a type identical to those in Figures 6 to 9, Plate 278, or a type represented in Figures 2 to 16, Plate 279, and which I had already obtained by culturing the pulp of rabid rabbit marrow. This identity can be appreciated for both Figures 14 and 15 of Plate 279 (*Penicilium* from rabid rabbit marrow from the Pasteur Institute) by comparing them to the other figures in the same plate (*Penicilium* form obtained by transformation of the *Aspergillus* form of the rabies mold isolated from the dog's brain).

2. A *Monosterigmata* form identical to that in Figure 4, Plate 278.

3. A *Cephalosporium* type identical to that in Figure 3, Plate 278

4. A *Corymbisporium* type identical to that in Figure 5, Plate 278.

These four gonidial forms were perfectly identifiable with those obtained previously. There was therefore no doubt to be preserved: the gonidial forms obtained (*Aspergillus, Penicilium, Monosterigmata, Acremonium, Cephalosporium, Verticilium, Corymbisporium*) were indeed those of the rabies pathogen.

By culturing on agar the brain and medullary pulp of rabid rabbits, inoculated with the fixed virus from the Pasteur Institute, these same gonidial forms are easily obtained.

Identification of the rabies pathogen.

I encountered a considerable difficulty at the beginning of this identification study, due to the possibility of confusing the rabies mold with the specific organic molds of dogs (Pl. 280 and 281) and rabbits (Pl. 282).

The organic mold of rabbits was eliminated by the fact that all gonidial forms of the rabies agent could be obtained by culturing the pulp of the nerve centers of dogs with spontaneous rabies.

The organic mold of dogs was eliminated by a study of differentiation with the rabies mold and, secondly, by the very fact of its identification.

The different gonidial forms of rabies mold are perfectly identified with the gonidial forms of carrot organic mold: we have already previously determined that this is the pathogenic agent of scarlet fever.

Both types of the *Penicilium* form are found in carrot molds, obtained either directly or by passage through the guinea pig's organism. The types in Plate 279 are identified with the types in Figures 4 to 14, Plate 260, obtained by direct transformation of carrot organic mold (carrot pulp cultured on agar). The types in Figures 6 to 9, Plate 278, are easily identified with those obtained by culturing the organs of guinea pigs inoculated with carrot juice.

As for the *Aspergillus* (Fig. 1, pl. 278), *Acremonium, Cephalosporium* (Fig. 3), *Corymbisporium* (Fig. 5), and *Monosterigmata* (Fig. 4) forms, they are perfectly identical to those obtained by direct or indirect transformation of the organic mold of carrots. They are obtained from cultures of the pulp of the organs of guinea pigs inoculated with carrot juice, or by culturing the pulp of the parenchyma of the latter on agar.

This identification, established by the study of the gonidial forms, is confirmed by the comparison of the bacterial cultures of the pathogenic agent of rabies with those of the pathogenic agent of scarlet fever on the one hand, and with those of the organic mold of carrot on the other.

The forms of the elements of the bacterial cultures of scarlet fever and carrot have been described and are shown in Plate 261. I used, as a term of comparison, bacterial cultures obtained from animals inoculated with the *Aspergillus* form extracted from the brain of a rabid dog. This mold is inoculated into guinea pigs. After death, the pulp of their organs is cultured on agar. In these cultures, a whitish material develops on the agar below the inoculated area; this material, consisting of the bacterial culture of the

inoculated mold, is inoculated into broth; the two cultures (agar and broth) are examined for comparison with the cultures obtained from carrots.

Bacterial cultures of carrots are obtained by two processes:

1. By culturing carrot pulp on agar, a creamy material develops, which is the bacterial culture.

2. By inoculating guinea pigs with carrot juice and cultivating the pulp of their organs on agar, a creamy, whitish material develops on it, which is the bacterial culture of the carrot.

It is necessary to have the cultures obtained by this latter process as well as the culture obtained by inoculation in broth.

The cultures of the rabies agent, obtained by passage through the guinea pig's body, can appear in three different forms:

1. In the form of oval masses 3 to 4 μ long by 2 to 2 μm wide. The culture also contains germinative masses or sclerotia which appear identical to the Negri corpuscles in the brain of rabid dogs.

Such a culture obtained in this form on agar reproduces in the same form on agar. After twelve to fifteen days, it begins to form some bacillary elements.

It is indeed a form of the rabies agent because, inoculated into a guinea pig, it reproduces the pure bacillary form; this transformation can even be obtained in artificial culture media.

I have not studied until now the origin and mode of formation of these oval elements.

2. In the form of round grains of various sizes (0 μ, 5 to 2 μ). I have not studied their mode of reproduction and formation. The culture of this form on agar reproduces in the same form in broth. In the long run, it ends up forming bacillary elements.

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3. In the form of bacilli. Agar cultures show bacillus of different lengths depending on their age; in young cultures, they are generally bacilli 0.4 to 0.7 μ wide by 1.5 μ long, sometimes 2 μ, which segment into two elements to form coccobacilli or into four elements to form granulations. In cultures aged five to ten days, certain bacilli progressively lose their basophilic protoplasm, of which they retain only two or three granules (polar bodies), then one, and finally become barely stainable. Certain bacilli are longer and wider (0.6 to 1 μ). These different aspects are found identically in bacterial cultures of carrot and scarlet fever (pl. 261).

A bacterial culture of the rabies agent is obtained by the following procedures:

1. By directly cultivating in broth a little cerebral matter from Ammon's horn or well-crushed and emulsified marrow.

2. The pulp of the cerebral or medullary substance of rabid animals being spread on agar, small, round bacterial colonies form there after a few days, which become opaque and very apparent as they grow. The creamy matter, constituting one of these colonies, being taken and inoculated in broth, quickly develops the bacterial culture of the rabies agent.

With marrow from rabid rabbits made available to me by the Pasteur Institute, I thus obtained the bacterial culture of the rabies agent constituting the fixed virus.

This culture is identical to that of the septic vibrio.

It is also morphologically identical to the bacterial cultures of the gonidial forms *Corymbisporium* and *Aspergillus* of the organic carrot mold; the bacterial cultures of these two forms *are themselves identical to that of the septic vibrio.*

Now, the anaerobic bacterial culture of the septic vibrio, transformed into a hyphomycete, gives rise to the gonidial form *Corymbisporium* of the organic carrot mold.

It is therefore the bacterial culture of the Corymbisporium form of the organic carrot mold that constitutes the fixed rabies virus, just as it is, on the other hand, the culture of the septic vibrio.

I indicated previously that a large bacterium, *identical to the septic vibrio,* swarmed in the organs of dogs suffering from street rabies or inoculated with the street virus.

It is therefore the same bacterial form, which vegetates in rabies animals, whether it is a street virus or a fixed virus.

The bacterial culture of the *Aspergillus* form of organic carrot mold also appears identical to that of the septic vibrio and that of the *Corymbisporium* form of carrot. This explains the rabies caused in dogs by inoculation with spores of the *Aspergillus* form from carrots.

* * *

It follows from these facts that the pathogenic agent of rabies is the organic sour mold of the carrot in one or more of its gonidial forms; we have already determined that carrots are the original source of the scarlet fever pathogen.

We are therefore led to conclude:

1. That street rabies in dogs, accompanied by congestion of the larynx, appears to be a form of scarlet fever infection in this animal.

2. That rabies is not a special, distinct disease, but only a form or cerebral complication of a contagious disease due to infection by one or more gonidal forms of the organic mold of the carrot.

If the bite of a dog or the subcutaneous inoculation of the virus cannot reliably reproduce nervous accidents (paralysis) or furious rabies, it is because it only determines the general infectious disease and can only reliably reproduce the local (cerebral) complication if the virus is brought to the very place where this complication occurs (intracerebral inoculation).

Rabies occurs in dogs in a wide variety of forms. It is currently being investigated whether this variety of evolving forms corresponds to an infection by various gonid forms of the carrot; the forms determined above (*Corymbisporium, Aspergillus, Vibrio septica*) in fact only apply to forms of furious and paralytic rabies. It has already been determined that the *Monosterigmata* form of the carrot determines a form of rabies evolving in approximately twenty-five days in dogs and ending with phenomena of paresis accompanied by muscle twitches.

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Other tests are being continued with the aim of:

1. To determine whether rabies can be induced in dogs by intracerebral inoculation of the blood of individuals suffering from scarlet fever that has only reached the beginning of the eruption phase, or has not yet reached this phase.

2. To determine whether injection of serum from individuals convalescing from scarlet fever can prevent the outbreak of rabies.

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CHICKENPOX

The pathogen of chickenpox, which remained unknown until now, is the organic mold of the tomato.

Some authors have argued that this agent is the same as that of smallpox. For Talamon, chickenpox, vaccinia, varioloid, and smallpox are simply different forms of the same disease and are caused by the same virus.

Currently, the fact that vaccinia and smallpox do not protect against chickenpox, and that chickenpox does not immunize against either smallpox or vaccinia, has led to chickenpox being considered a separate disease caused by a different virus than smallpox.

In a long series of researches, I isolated and then identified the smallpox virus: the source of this virus, as well as that of the cowpox virus, is the organic mold of the potato. This conclusion was reached, despite the failures of vaccination trials with potato pulp inoculations, because of the multiplicity of evidence of identity between the gonidial forms of the cowpox and cowpox molds and those of the organic mold of the potato.

Research to identify the chickenpox agent confirmed the nature of the variola virus; it was likely that the chickenpox virus, although distinct from the smallpox virus, must nevertheless have some relationship with it. Indeed, research to identify the chickenpox virus demonstrated that its source was the organic mold of the tomato. Now, the tomato is a nightshade like the potato, and its flowers and leaves bear a strong resemblance to those of the tomato. This therefore confirmed the accuracy of the identification of the smallpox virus.

Ten cases of chickenpox were studied to achieve the isolation of the pathogenic agent, which I obtained in all cases. For the research, the scabs of the pustules and the lymph that can be removed from them before the scabs are removed are used. The scabs are applied by their epidermal side to agar or carrot; the lymph is cultured in broth. Various molds develop on the scabs; often only a creamy, bacterial material develops, which flows onto the culture medium.

The lymph, which is always obtained in very small quantities, must be inoculated in a small quantity of broth, 1 or 2 cubic centimeters; when the culture is developed, it is transferred to a matrass. This, like the initial culture, being kept at 22-24°, sometimes develops in the long run, either a veil of mold, or floating mycelial colonies which, upon reaching the surface, give aerial fruiting bodies. To quickly obtain gonidian forms of the pathogenic agent, it is preferable to inoculate the culture in the guinea pig and to cultivate the pulp of its organs on agar. We thus easily obtain the *Penicilium, Aspergillus, Sterigmatocystis, Monosterigmata* and *Sporothricum* forms, and a copious, vigorous bacterial culture, in the form of creamy material which flows in the tubes. These various elements are sufficient for identification.

I thus obtained the following gonidial forms of the pathogenic agent:

1. The *Monosterigmata* form (Fig. 3, pl. 262).
2. The Sterigmatocystis form (Fig. 2, 4, 5, pl. 262).
3. The *Penicilium* form (Fig. 1 and 6 to 10, pl. 262).

The culture of chickenpox scabs very easily gives rise to the *Sterigmatocystis* form, more rarely to the *Monosterigmata* form, and, with difficulty, to the perfect *Penicilium* form.

To obtain the latter at will, I had to inoculate bacterial cultures of chickenpox, or lymph from pustules, into guinea pigs and cultivate the pulp of their organs on agar.

Bacterial cultures of chickenpox in broth or on agar often develop in the form of spherical grains, isolated or joined together in pairs, and reproduce in the same bacillary form. They are more easily obtained by culture on agar of the pulp of the organs of inoculated guinea pigs.

Figs. 7, 8, 9, and 10 of Plate 206 represent the different forms that the pathogenic agent of a case of chickenpox, the first that I studied, can take. I obtained a first culture whose elements are joined grains in the form of diplococci (Fig. 8), a second in the bacillary form (Figs. 9, 10), and a third of a characteristic form containing, in addition to diplococci, large oval masses reproducing by budding (Fig. 7).

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Identification of the pathogen.

Faced with the failures of my attempts at immunization against cowpox using potato tuber pulp or the bacterial cultures that can be obtained from it, I investigated whether the smallpox virus might not be the organic mold of a very similar plant; I therefore made cultures of plants from the same family, the tomato and the eggplant, to obtain the bacterial cultures and the gonidial forms.

From the first tomato cultures obtained, I noted the perfect identity of one of them with the chickenpox culture represented in Figure 7. I noted an equally perfect identity between a bacillary culture of tomato and the chickenpox culture represented in Figs. 9 and 10 (Pl. 206).

The chickenpox virus must therefore have been the organic mold of the tomato. Cultures of tomato pulp easily gave rise to the gonidial form *Sterigmatocystis*, but with difficulty to the *Penicilium* form. A guinea pig was inoculated with tomato pulp and the pulp of its organs cultured on agar. The *Penicilium* form obtained was identical to that of chickenpox. Identification was therefore carried out with certainty by bacterial cultures and by the two gonidial forms *Penicilium* and *Sterigmatocystis*; new gonidial forms are being sought to complete it.

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COWPOX, VACCINIA, SMALLPOX, AND FOOT-AND-MOUTH DISEASE

I have grouped these three diseases together because they are only different forms or aspects of the same disease that evolves either in the form of smallpox when it is spontaneous, or of vaccinia when it is conferred by vaccine inoculation, or in the form of foot-and-mouth disease in cattle or other animals. Cowpox in cows is a form of sponta-

neous foot-and-mouth disease; this conclusion is forced by the fact that the vaccinia virus (which is cowpox) has the same original source as foot-and-mouth disease.

The original source of the pathogens of these various forms is the same: organic mold on the potato. But each disease is distinguished from the others by the quality of the infective gonidial form.

These conclusions do not result from more or less plausible hypotheses, but from demonstrations by positive facts so convincing that the slightest doubt can exist. Indeed:

1. I transformed the active agent of the vaccine, which is a mold, into three different gonidial forms: *Penicilium, Verticilium,* and a third of the *Ovularia* form. These three gonidial forms are characteristic of the organic mold of the potato to which they belong. *Verticilium* is the species *Verticilium alboatrum*.

2. I isolated, using the crusts of smallpox pustules, the mold that is the pathogenic agent of smallpox in its bacterial and gonidial forms. Among the latter, I obtained:

A. *Phytophtora infestans* or *Peronospora solani* and its evolutionary form or *Mucor* type.

B. *A Passalora* form

C. *Spicaria solani.*

D. *Aspergillus solani.*

E. *Sterigmatocystis solani.*

F. *Penicilium solani.*

G. *Monosterigmatis solani.*

H. An intermediate form between the *Monosterigmata* form and the *Spicaria* form.

All these gonidial forms are characteristic of the organic mold of the potato.

3. I isolated from the lymph of the canker sores the mold that is the pathogen agent of bovine foot-and-mouth disease in its bacterial and gonidial forms. I obtained from the latter:

A. The *Monosterigmata* form, identical to that of the variola mold and that of the organic mold of the potato.

B. A distinct gonidial form, intermediate between the *Spicaria solani* form and the *Verticilium alboatrum* form. I obtained this same gonidial form by direct transformation of the organic mold of the potato.

C. Verticilium alboatrum or Verticilium solani.

D. Fusarium solani.

E. Penicilium solani

4. The characteristic elements of the bacterial cultures of vaccinia, smallpox, and foot-and-mouth disease are identical to those of the bacterial cultures of potato mold or its gonidial forms.

This study presented the greatest difficulties, on the one hand because of the rarity of the smallpox cases that I was able to study (only two) and, on the other hand, precisely because of the common origin of the three diseases. The search for the identification of their specific molds and the original source of the virus gave a conclusive result for foot-and-mouth disease in the first place; knowing that this disease is caused by potato mold, I believed for a long time that it could not also be the agent of vaccinia and smallpox. But the simultaneous and prolonged study of the bacterial and gonidial forms of the active agent of these diseases finally led to their identification.

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VACCINIA

I studied vaccinia using vaccine pulp and glycerin vaccine, which Dr. Camus, director of the Vaccine Institute of the Academy of Medicine, kindly made available to me on numerous occasions.

The gonidial forms of vaccine mold are easily obtained by various methods:

1. By spreading the previously crushed vaccine pulp with a little distilled water on agar and sugared carrot. The culture tubes are kept at laboratory temperature (16-18°C) or in a warm room (22-24°C). The mold colonies that develop are transferred to other culture tubes as soon as they have reached sufficient development.

2. By inoculating the guinea pig with a little vaccine pulp or glycerin vaccine and culturing the organ pulp (lung, liver, spleen) on agar, after the animal's death.

As for the bacterial culture, it is obtained without difficulty by inoculating glycosylated peptone calf broth with a little vaccine pulp or glycerin vaccine, the cultures being preserved at room temperature (18-20°) and in light, which does not appear to hinder their development in any way; it also exists in the form of a creamy coating that runs to the bottom of the tubes, in cultures of the organ pulp of animals inoculated with the vaccine.

Before beginning the study of the forms of vaccine mold, it is necessary to recall that I obtained the gonidial forms of organic potato mold by spontaneous, natural transfor-

mation of the latter using appropriate culture methods. I thus obtained the forms *Phytophtora, Mucor, Monosterigmata, Verticilium, Spicaria, Fusarium, Penicilium*, etc., and, in addition, the bacterial culture with its various forms. These forms, or at least most of them, are represented in Plates 145 and 149, which will be necessary to refer to for the identification of the types.

Gonidial forms of vaccine mold.

By culturing heifer vaccine pulp on solid media, I obtained four different gonidial forms: *Penicilium, Aspergillus, Verticilium,* and one form that I provisionally assign to the type *Ovularia* (Saccardo) to designate it.

I have eliminated the *Aspergillus* form, the identification of which has been insufficient so far. On the other hand, the three other gonidial forms belong to the organic mold of the potato.

Ovularia form

The first form, referred to the *Ovularia* type (Fig. 1, 2, pl. 263), is characterized by large gonidia or oval masses borne laterally at the end of the mycelial branches. An identical form has been obtained in potato cultures (Fig. 7, 8, 9, 10, pl. 147); it is observed especially on bacterial cultures of potatoes inoculated on agar (rotten potato), which evolve into the *Fusarium* type or the *Penicilium* type. It also develops directly, in a very similar form, on the pulp of the aerial stem of a healthy potato spread on agar (Fig. 6, pl. 146). This gonidial form is a stage in the evolution of the mold towards the *Penicilium* or *Citromyces* forms.

To the already sufficient identification of this gonidial form of the vaccine mold was added another proof; this form was transformed spontaneously, in the culture itself, into a *Penicilium* form which was *Penicilium solani.*

Verticilium form

The second gonidial form of vaccine mold (Figs. 3, 4, pl. 263) is the potato organic mold *Verticilium alboatrum.* Identifying this type was difficult. Indeed, the beet organic mold has a *Verticilium* form that is almost identical and so similar that I initially believed, and for quite a long time, that the original source of the vaccine virus was beet; but I obtained, by inoculation of the rabbit and culture of its organs, the transformation of the *Verticilium* form of vaccine mold into *Penicilium solani* (Figs. 1 and 3 to 9, pl. 264). Finally, as definitive proof, I obtained the *Verticilium solani* form similar to that of vaccine mold, in cultures of the pulp of the organs of a guinea pig inoculated with juice from the parenchyma of a potato tuber.

Finally, the *Verticillium alboatrum* crop has a darker reddish tint than that of the beetroot *Verticillium*, which is yellowish ochre or pale reddish ochre; this color difference is enough to distinguish the two crops. This is therefore indeed Verticillium solani.

Penicilium Form

The *Penicilium* form of vaccine mold is very easily obtained in cultures. Whether directly (spreading the vaccine pulp on agar) or indirectly (inoculation of the guinea pig and culturing the organs), the cultures become completely covered with green *Penicilium* colonies. Identification of this *Penicilium* form is generally complicated by the fact that cultures grown directly or indirectly from vaccine pulp have a vigorous, broad mycelium, resulting in the gonidial arborizations having a heavy, pasty appearance, which modifies their appearance; nevertheless, the essential characteristics remain unchanged. Identification will be facilitated by choosing, as terms of comparison, the types of a *Penicilium* culture obtained by culturing the organs of a guinea pig inoculated with a little crushed potato pulp or juice.

Limited in this book by the excessive number of plates, the comparison will be made here with the types of plates 272 and 273, chosen from two spontaneously developed *Penicilium* cultures, one (Pl. 272) on healthy potato pulp spread on agar, the other (Pl. 273) on the parenchyma of a potato tuber broken into two pieces and preserved in a sterile crystallizing dish.

Plates 264 and 265 show different types of the *Penicilium* form of the vaccine mold; those in Plate 265 are common and difficult to identify; however, their identification can be achieved by comparing them to those of a *Penicilium* culture resulting from a direct transformation of *Fusarium solani*: this contains, in particular, numerous fruiting bodies identical to the types in Figures 6 and 7 of Plate 264, developed spontaneously on rabbit vaccine pulp cultured on agar.

At the same time as this comparison of the *Penicilium* form of the vaccine mold with that of the organic potato mold, another comparison must be made with that of the smallpox and foot-and-mouth molds.

From this comparison results:

1. A conclusion of identity between the vaccine, smallpox, and foot-and-mouth molds.

2. The identification of these molds with the organic mold of the potato.

This identity, which results from the sole comparison of the *Penicilium* form of pathogenic molds with *Penicilium solani,* is further confirmed by the fact that the *Verticilium alboatrum solani* form is a gonidial form belonging to both the agents of foot-and-mouth disease and vaccinia.

Bacterial forms of vaccinia mold.

The methods by which they can be obtained have been indicated; they must be made and stored at a temperature of 18-24°. Plate 266 shows:

1. The form of the elements of a 24-hour broth culture obtained by inoculating a bacterial colony developed on the liver pulp of a guinea pig inoculated with a little vaccinia pulp (Fig. 1 and 2).

2. The shape of the elements of a vaccine pulp culture in glycosylated peptone calf broth, after twenty-four hours (Fig. 3), six days (Fig. 4), nine days (Fig. 5) and thirteen days (Fig. 6).

3. The shape of the elements of a glycerin vaccine culture in broth after two days (Fig. 7) and thirteen days (Fig. 8).

Examination of these figures reveals that there is a variety of bacterial forms in these cultures, such that an uninformed observer would conclude that there is a bacterial association, that is, a mixture of different species. It is probably this variety of forms that has so far confused observers in the study of vaccinia bacterial cultures. In reality, there is no bacterial mixture, but bacterial polymorphism in a pure culture of a single species.

The reason for these multiple and very different bacterial forms in vaccinia cultures becomes apparent as soon as one examines the bacterial cultures of the potato; one immediately observes the same polymorphism and the same bacterial forms. It is unnecessary, for this comparison, to refer to special broth cultures.

Indeed, the natural bacterial cultures that arise spontaneously inside a potato, by transformation of its organic mold under various conditions, are perfectly identical to vaccinia cultures. This question of spontaneous cultures in potato tubers has already been treated elsewhere; the bacterial forms observed are shown in Plate 145; they have five different origins.

1. Figures 1 and 2 represent the bacterial elements of natural potato rot in the soil; they show two categories of elements: small ones and much larger ones. The thin

bacilli are 0.2 to 0.4 microns wide; the large bacilli are 1 to 10 microns. The latter are the segmentation elements of the organic mold which, evolving into the *Phytophtora* type, does not succeed and resolves into a bacterial culture. These segmentation elements are found in the mycelium of *Phytophtora infestans.*

2. A simple method for obtaining a pure bacterial culture of the potato is to wash and brush it thoroughly, sanitize the exterior, and then subject it to freezing by placing the aseptic vessel containing it in a mixture of ice and sea salt. Removed from this mixture and kept at room temperature, it rots and releases a liquid which is a pure bacterial culture of the potato (Fig. 3, pl. 145). This culture contains a large bacillus form 1 µm wide and a smaller polymorphic form (0.2 to 0.5 µm wide) with intermediate elements between the two sizes. The rot of the tuber itself is very rich in large elements (1 to 10 µm, fig. 4, 5, pl. 145).

3. Another process consists of destroying the organization of the tuber by heat. After having sanitized it, it is heated for an hour or two at 55° in sterile water. The tuber thus treated, and left to itself, rots like the frozen tuber and oozes a liquid which is a pure bacterial culture of the potato (Fig. 6, 7, pl. 145); in this culture we find elements identical to those of the culture obtained by freezing.

4. A bacterial culture can be obtained by aseptically scraping the parenchyma from a tuber section and spreading the pulp on agar. A bacterial culture is formed in the form of a thick, semi-liquid substance that flows to the bottom of the tube. This bacterial culture, examined at the beginning of its formation, appears to be composed of elements identical to those of the vaccinia bacterial culture represented in Figures 1 and 2 of Plate 266.

A culture is also obtained by cultivating the pulp of any other part of the plant, such as a stem or fruit, on agar. Figure 8, plate 145, represents the form of the bacterial elements of a culture of the fruit's bacteria on agar, subcultured in broth after twenty-four hours.

5. Finally, a bacterial culture can be obtained by the return of the gonidial forms of the mold to the bacterial state; figures 9, 10, 11, 12, plate 145, represent the bacterial elements of a pure culture of *Fusarium solani, spontaneously liquefied* and still forming, in addition to bacterial elements, rudimentary (bilocular) fruiting bodies of *Fusarium.*

Comparison of the bacterial forms of vaccine mold with those of organic potato mold demonstrates their identity; the bacterial forms of vaccine mold, contained in Figures 3, 4, 5, 6, of Plate 266, are identical to those of organic mold of frozen or heated potato contained in Figures 3, 6, 7, 8, of Plate 145.

The large bacillary form developed alone in the glycerin vaccine culture in broth is that of the potato cultures apparent in Figures 2, 3, 4, 5, 7, of Plate 145; it developed alone in a pure state under a special influence which, probably, is that of glycerin.

Thus, the identity of vaccine mold and organic potato mold, established by the comparison of their gonidial forms, is confirmed by the comparison of the bacterial forms.

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SMALLPOX

Since vaccinia immunizes against the acute or bacterial phase of smallpox, it follows that the latter must also be caused by the organic mold of the potato and that, consequently, *all the gonidial forms of the variola mold must be those of the potato, otherwise the demonstration which has just been made for vaccinia would be false.* We will see that the study of smallpox has given perfect confirmation of the origin of vaccinia by the knowledge it provides of six gonidial forms of the variola mold which are characteristic of the potato.

I was able to obtain variola virus from two cases of smallpox. From the first, hospitalized at the Marseille lazaretto, a dozen large pustule scabs were sent to me by Doctor Ribot, the hospital's physician. I owe, to the kindness of Doctor Valleteau de Mouillac, physician at the Cherbourg hospital, materials from a second case of smallpox developed in a black man, a cook aboard a transatlantic liner; from this case, I had at my disposal about sixty scabs, that is, a quantity of virus sufficient for a long study.

From these scabs, I obtained the pathogenic agent of smallpox in its gonidial and bacterial forms; they were cultured on carrots and on agar, the external cutaneous side applied to the surface of the culture medium.

From these cultures, eight gonidial forms were obtained, either directly or by indirect transformation of the *Passalora* and *Aspergillus* forms; the first six were obtained using the scabs of the patient from the Marseille Lazaret, the last two with the scabs of the patient from Cherbourg; these forms are:

1. A *Penicilium* form (Fig. 4 to 14, pl. 270).
2. A *Citromyces* form (Fig. 5, 6, pl. 268).
3. A *Spicaria* form (Fig. 7, 8, pl. 268).
4. An *Aspergillus* form (Fig. 1, 2, pl. 268).
5. *Phytophtora infestans* or *Peronospora solani,* which developed on cultures of the pulp of the organs of a guinea pig inoculated with the *Aspergillus* form.

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6. A gonidial form that I provisionally assign to the *Passalora* type (Fig. 3, 4, pl. 268).

7. Monosterigmatis solani.
8. Sterigmatocystis solani.

Penicilium form.

This form is *Penicilium solani;* a comparative study of the types demonstrates the identity:

A. Of the variolic *Penicilium* obtained by the direct route (culture of the scabs of the pustules (Fig. 4 to 14, pl. 270)).

B. Of the vaccinal *Penicilium* (Plates 264 and 265).

C. Of the *Penicilium* of the foot-and-mouth disease mold (Fig. 1 to 7, pl. 276).

D. Of the *Penicilium* of the organic mold of the potato (Pl. 272, 273).

Citromyces form

The *Citromyces* form (Fig. 5, 6, pl, 267) is also identical to that of the organic mold of the potato.

Spicaria form.

The *Spicaria* form of the variola mold is *Spicaria solani,* an identification that can be easily verified by comparing Figures 7 and 8 of Plate 267 (smallpox) with Figure 7 of Plate 146 (*Spicaria solani,* developed spontaneously in a culture of healthy potato fruit pulp).

The culture of the smallpox *Spicaria* is, like that of *Spicaria solani,* reddish-ochre in color; the identification was completed by a study of gonidial transformation; the spores of the variola *Spicaria* were inoculated into a guinea pig whose organ pulp was cultured on agar after death. The cultures became covered with *Penicilium solani.* The identification was therefore perfectly secure.

Aspergillus and Phytophtora infestans forms

This form (Fig. 1, 2, plate 267) is that of *Aspergillus solani*. It is easily obtained in cultures of the pulp of the organs of a guinea pig inoculated with potato tuber juice; it

appears either with short, stocky gonidiophores, as in figures 1, 2, plate 267(smallpox), or with long gonidiophores; the two types can be confused in the same culture.

The identification of this *Aspergillus* form was further sought by transformation. To this end, spores from an *Aspergillus* culture emulsified in salt water were inoculated into two guinea pigs. In cultures of the pulp of the animals' organs made after death, colonies of the most characteristic gonidial form of the potato developed: *Phytophtora infestans.* This mold was obtained in two forms:

One was the characteristic form with large oval gonidia (Fig.4, 5, pl. 268); it evolved into the *Mucor* type (Fig. 6, 7, pl. 268), as often happens with *Phytophtora infestans*; Some of the mycelial filaments had the characteristic form of the developing mycelium of *Phytophtora* (Fig. 2, plate 268. Compare Fig. 1, plate 147: spontaneous development of *Phytophtora* on potato rot). Other mycelial filaments, the most numerous, presented the characteristics of the mycelium of *Mucor solani* and *Phytophtora* (Fig. 1, 4, 5, 6, 7, plate 268 (smallpox) and Fig. 4, 5, 6, plate 149 (potato)). I recall that this *Phytophtora* form, represented in Figures 6, 7, 8, plate 149, was obtained by spontaneous transformation of the healthy organic mold of the potato (Culture of pulp from the center of a tuber on agar).

The other is the form of *Phytophtora* (Fig. 3, plate 268) that I obtained by spontaneous development on the pulp of the center of the healthy stem of the potato grown on agar (Figs. 1, 2, 3, plate 146); it is a lean form, quite poor in gonidia.

The identification of the *Aspergillus* form of the variola mold was therefore perfectly safe, since it transformed into *Phytophtora infestans,* the most characteristic gonidia form of the potato with *Fusarium solani.*

Passalora form.

This gonidia form of the variola mold is represented in Figures 3, 4, plate 267. I also obtained it from the organic mold of the potato, by culturing the pulp of the organs of a guinea pig inoculated with tuber juice. The mold obtained, not shown in the plates, had

the characteristic oval, pedunculated balls of *Passalora*, but there were only a few of the characteristic shape; the others all evolved into the special type of *Sterigmatocystis* form, whose sterigmata emit remarkably long chains of spores.

The identification of the mold developed on smallpox scabs was definitely achieved by indirect gonidial transformations. An emulsion of a culture of the mold was inoculated into the rabbit, whose organ pulp was cultured on agar after death. On the liver pulp, the *Aspergillus* form (Fig. 1, 2, pl. 269) developed, already obtained directly by culture of the scabs (Fig. 1, 2, pl. 267); On the pulp of the lung, a *Penicilium* form developed (Fig. 3 to 8, pl. 269 and fig. 1, 2, 3, pl. 270) which is the light or slender form of *Penicilium solani* (Compare to fig. 4, 5, 6, 7, 11, 12, 13, 14, 15, 16, pl. 273, potato).

Thus, this special form, which has remained stable until now, is indeed a gonidial form of the organic mold of the potato.

The six gonidial forms that we have just studied were obtained by transformation of the same variola virus from the smallpox case of the Marseille lazaretto.

Among these six gonidial forms, three are characteristic of the potato and easily identifiable: these are the *Phytophthora infestans, Spicaria solani,* and *Penicilium solani* forms. The others can be identified with certainty by transformation.

This set of gonidial forms of the variola mold therefore constitutes a sure demonstration of its nature and origin: it is the organic mold of the potato; this plant is therefore the original source of the variola virus.

The study of the second case of smallpox from the Cherbourg hospital confirmed this conclusion: the culture of the scabs directly provides three gonidial forms: *Penicilium solani, Sterigmatocystis solani*, and an evolved *Monosterigmata* form.

The *Penicilium* form (Figs. 1 to 12, pl. 271) was that of *Penicilium solani*.

Monosterigmata form

This form represented in figures 13, 14, 15, pl. 271, is the *Monosterigmata* form of organic mold of the potato.

The comparison between the two molds shows complete identity. This same *Monosterigmata* form is also obtained by transformation of the foot-and-mouth disease virus and by transformation of tetanus cultures. The two molds thus obtained identify perfectly with the *Monosterigmata* form of the organic mold of the potato.

Sterigmatocystis form

This gonidial form identifies with that of the potato. It is obtained directly on smallpox scabs cultured on agar, but rather in cultures of the pulp of the organs of guinea pigs inoculated with an emulsion of smallpox pustule scabs crushed in water.

In these cultures, the *Sterigmatocystis* form develops in two forms:

1. In the form of a rounded, protruding colony, light meadow green in color in the center, then yellowish and finally creamy white around the edges; the color of the colonies varies; they may be barely tinged with greenish yellow in the center; the fruiting head is globular, and the fine spores emitted by the sterigmata are not linked in chains, or only in very short chains.

2. In the form of a milky white colony, not very protruding. The fruiting heads, which are often fan-shaped, emit large spores which remain united in very long chains, adhering to the sterigmata for a long time. This very special form comes from the transformation of the *Passalora* form.

These two *Sterigmatocystis* forms, perfectly identical to the previous ones, were obtained in cultures of the organ pulp of the guinea pig inoculated with potato tuber juice. The white form generally develops late in the cultures.

The two new gonidial forms, obtained from the scabs of the second case of smallpox studied, therefore confirm the identification already established previously.

The pathogenic agent of smallpox is therefore the organic mold of the potato.

Let us now study the bacterial cultures of the variola virus to see if they identify with those of the organic mold of the potato.

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Obtaining and Studying Smallpox Bacterial Cultures.

The bacterial forms of smallpox are obtained:

1. By direct culture of the pustule scabs in glycosylated veal peptone broth, stored at room temperature (18-24°C).

2. By inoculating the guinea pig with either an emulsion of crushed pustule scabs or gonidial forms of the variola mold, and culturing the organ pulp on agar after death. On these cultures, a semi-liquid creamy material forms, either in colonies or as a layer, which is a bacterial culture of the variola agent.

The forms represented in Plate 274 were all obtained by culturing the organs of the guinea pig or rabbit inoculated either with the *Aspergillus* form of smallpox, or with the *Phytophtora* or *Mucor* form obtained from this *Aspergillus.* The *Phytophtora* culture was chosen, in particular, because, by liquefying in the agar tubes, it emitted a fetid odor identical to that of smallpox.

The spores of the gonidial form of *Aspergillus*, inoculated into the guinea pig intravenously, develop the bacterial culture shown in Figure 1, Plate 274; all the other figures in this plate (Figs. 2 to 8) relate to the bacterial types existing in agar cultures of the pulp of organs of the guinea pig or rabbit inoculated with the *Phytophtora* form derived from *Aspergillus* grown directly on smallpox scabs; in these cultures, transferred to broth, two bacillary types coexist, from the first to the eighth day: a large, squat type, 0.5 to 1 µm wide, and a thinner type, 0.3 to 0.5 µm on average; the bacilli of both types vary in length depending on the degree of segmentation; but the squat type is most generally 4 µm long on average (3.5 to 4.5 µm).

These two types are found in all cultures. *These are the two types that have already been reported in bacterial cultures of vaccine mold* (Fig. 3, 5 and 7, pl. 266) *and in bacterial cultures of organic potato mold* (Fig. 3, pl. 145).

In addition, in cultures from one to eight days old, we find either agglomerated masses of various sizes, often grouped in clusters (Fig. 2, pl. 274), or large mycelial trunks emitting branches which, by segmentation, release the bacilli (Fig. 5, pl. 274).

In cultures eight days old or more, whose development does not stop at these bacillary forms and which tend to evolve towards the reconstitution of aerial hyphomycete forms, we find, in addition to the mycelium which segments into bacilli of the stocky type, another form of this same mycelium, also segmented, but which loses its basophilic protoplasm (by emission of basophilic elements to the outside) and transforms into long chains of coccobacilli provided with two polar globules (Fig. 6, 7, 8, pl. 274). Figures 7 and 8 show, in addition to these long chains, filaments segmented into bacilli of the stocky type in the process of transformation into the coccobacillary type.

In addition to these chains of coccobacilli, cultures older than 8 days, whose evolution continues, also contain a new, thin bacillary form (1 to 2 μm wide), of variable length, very small coccobacilli and granulations (Micrococci, Figs. 6, 7, 8, pl. 274). These latter elements exist in an identical form in the wet rot of the potato (Figs. 1 and 2, pl. 145) and in the old bacterial cultures of the organic mold of this plant; they also exist in the old cultures of foot-and-mouth disease in broth (Fig. 14, pl. 276); in the latter, they coexist with the other bacillary types, including the stocky type.

In summary, the bacterial cultures of the variola virus do indeed contain the same elements as those of the vaccinia virus and the organic mold of the potato. However, the bacillary elements of the variola virus are generally larger than those of the vaccinia virus. Furthermore, the shape of the culture elements represented in Figures 5 to 8 of Plate 274, particularly the chains of bacilli and coccobacilli, appears to clearly differentiate smallpox from vaccinia. Following a series of new research, I had an explanation for these differences: they result from the fact that *the elements of the bacterial cultures of the organic mold of the potato are different, depending on the gonidial form that gives rise to them.*

The large bacillary elements represented in Figure 1, the chains of bacilli and coccobacilli represented in Figures 6, 7, and 8, are the elements of bacterial cultures of the potato *Aspergillus* form. This is obtained by inoculating a pure culture of *Aspergillus* into broth, and taking care to scrape the surface of the agar with a spatula at the point where the sample is taken; in fact, pure spores hardly give a bacterial culture; on the

contrary, the segmental elements and the zymospores of the mycelium of *Aspergillus,* which swarm on the agar, easily give rise to the bacterial culture in broth. Having obtained such a culture, I noted that it gave chains of large bacilli and other chains of coccobacilli identical to those of figures 6, 7, 8, plate 274; as they age, the bacilli gradually lose their basophilic protoplasm and present clear zones which are seen in a bacillary chain of figure 8, plate 274.

Bacilli do not always have the same size in cultures; we must remember that constancy and invariability are properties that molds lack. Also the culture represented in Figure 5, Plate 274, which is however a bacterial culture of the *Aspergillus* type, shows bacilli which are of a smaller width than that of the large bacillary form of Figure 3 which is more characteristic of the *Aspergillus* type. Let us note here that this bacillary culture is identical to that which I obtained by transformation of anaerobic tetanus cultures; these are transformed on agar into a creamy, light yellow material, which is a bacterial culture of the *Aspergillus* type, the bacterial culture of *Aspergillus solani* takes, on agar, the same appearance of a creamy, light yellow material.

This explains the differences that can be observed between various cultures having a common original source.

These facts allow us to return to an identical difference observed in vaccinia cultures. The bacterial forms developed in cultures of the pulp of the organs of a guinea pig inoculated with vaccinia pulp are, for example, those contained in Figures 1 and 2, Plate 266, while a direct culture of the same glycerinated vaccine in broth will contain the bacterial form of Figure 7 of the same plate; the former forms are observed in direct cultures of the pulp of a potato tuber or stem; the latter are those of the *Aspergillus* form of the potato.

In summary, the bacterial cultures of vaccinia, smallpox, and potato show great variability; but since the cause of this is known and the gonidial origin of the cultures is determined, the different bacterial forms of vaccinia and smallpox can be identified with those of the organic mold of the potato. The study of smallpox bacterial cultures therefore confirms the established identification.

Control of the identification of vaccine and smallpox molds by studying their vaccinating action.

Having established that the potato is the common original source of the pathogenic agents of cowpox and smallpox, I found myself in great difficulty in confronting my conclusion with the following historical knowledge:

1. Smallpox already existed in the ancient continent in the most remote times, since the principle of smallpox vaccination was known and applied more than three thousand years ago!

2. The potato would have existed in the ancient continent for only about three and a half centuries, having been imported from America, it is said, around the year 1586.

Now, since smallpox wreaked terrible havoc in Europe in the twelfth century, for example, the potato could not be the cause if it was not imported from America until 1586!

I therefore had to check the identification of the molds obtained using the vaccine and variola viruses several times, and each time, the conclusion was reinforced. These viruses do indeed give rise to the most characteristic gonidial forms of the potato, *Verticilium alboatrum, Spicaria solani*, etc.

The result of this repeated check was to demonstrate to me that, while my conclusions were correct, historical knowledge was not. The potato must always have been cultivated in the ancient continent, and this since the most remote times. Research is currently being conducted to find traces of this cultivation before the sixteenth century. Other research, relating to the reconstitution of the smallpox vaccine using the potato itself, will, if successful, constitute definitive proof.

My first tests, relating to the possibility of vaccinating with molds from the vaccine and variola viruses, led to the following observations:

1. Inoculation of the rabbit with the variola culture, the elements of which are shown in Plate 274, does not immunize the rabbit against the action of the vaccine.

2. Inoculation of the *Penicilium* form of the variola mold does not immunize against the effect of the vaccine.

However, in one case, I achieved complete immunization in a rabbit on the thirty-second day after inoculation with the Penicilium form shown in Figures 3 to 8 of Plate 269.

3. Inoculation with the Penicilium and Verticilium forms of vaccine mold does not immunize against the effects of vaccinia.

Verifying the effect of vaccine and variola molds is difficult because they are very virulent and the animals often die before control vaccination can be attempted; however, it can only be attempted late.

The case cited above shows that immunization can develop, but it is exceptional. Moreover, once the identification of vaccine and variola molds was established, I abandoned the search for their vaccine properties to focus solely on the study of the vaccine or variola-promoting properties of organic potato mold.

From the very first tests, I observed that inoculating the rabbit with potato tuber juice, or the bacterial culture it develops in broth, does not immunize the rabbit against the effects of the vaccine; twenty-five days after inoculation, the control vaccine applied to the animal's back develops the characteristic vaccine pustules.

Should we conclude from this negative result that the identification of the vaccine and smallpox molds was inaccurate? *The elements of this identification were verified several times, and each time this new examination confirmed the initial determination.*

It is indeed impossible not to conclude that the gonidial forms of the vaccine and smallpox molds are identified with those of the organic potato mold when we note:

1. That vaccine and smallpox are linked together, since they exert a reciprocal vaccinating action against each other.

2. That the vaccine virus gives rise, by transformation, to two gonidial forms of the potato, *Penicilium solani* and *Verticilium solani.*

3. That the variola virus gives rise, for its part, by transformation to other gonidial forms of the organic mold of the potato: the forms *Phytophtora infestans, Aspergillus solani, Spicaria solani, Passalora solani, Monosterigmata, Penicilium, Citromyces*, and *Sterigmatocystis solani.*

4. That the bacterial cultures of vaccinia are identical to those of the potato.

5. That the isolation and identification of the agent of chickenpox, a disease very similar to smallpox, makes it known that it is due to the organic mold of a plant very similar to the potato, the tomato, belonging to the same family.

These facts constitute a clear, evident demonstration of the common original source of the vaccinia and variola viruses; this source is the organic mold of the potato.

Since the inoculation of the formed elements contained in the parenchyma of the potato, or of those of the bacterial culture that they develop in broth, does not vaccinate, although they are very virulent, *it therefore becomes certain that it is not in this form that they constitute the viruses of vaccinia and smallpox.* I had obviously looked in the wrong direction.

This conclusion could, moreover, be based on known facts.

Indeed, the *Aspergillus* form of the rat's organic mold causes icterohemorrhagic spirochetosis. The *Penicilium* form of the same mold is almost as virulent, but no longer causes the same lesions; inoculation with an emulsion of rat brain substance kills the guinea pig in only about three weeks, while the *Aspergillus* and *Penicilium* forms kill it in eight to ten days; the lesions are also very different.

The two *Aspergillus* and *Penicilium* forms of brewer's yeast have different fermentative properties. The *Aspergillus* form should therefore not, because of this difference, vaccinate against the *Penicilium* form.

It is therefore not surprising that only one of the gonidial forms of the potato's organic mold can cause the vaccine pustule and that the other forms of this mold have no protective action against the effects of the vaccine or the variola virus.

Here was a group of phenomena whose determinism I had not yet addressed. New research was therefore undertaken:

1. To determine whether a gonidial form of organic mold from a plant or animal vaccinates against other gonidial forms coming from the same source.

2. To determine which is the gonidial form of Organic potato mold capable of creating vaccine immunity in rabbits by inoculation.

3. To determine whether the gonidial form of the organic potato mold that causes smallpox is different from that which causes vaccine eruption.

This research is currently underway and will be the subject of a new publication.

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FOOT-AND-MOUTH DISEASE OF BOVINES

The following study was carried out with the serosity of the mouth ulcers of bovids suffering from foot-and-mouth disease; in two cases, this serosity was taken from the mouth ulcers of the tongue; in the others, it was taken from the hoof ulcer. In these latter cases, I was able to obtain a large quantity of pure, uncontaminated serosity.

The lymph was also collected at various times of the year and in locations far apart from each other. A bacterial culture and several gonidial forms were obtained from the lymph of each case. The different cultures from lymph of various origins were all recognized as identical; the gonidial forms obtained were related to a single mold: the organic mold of the potato.

The pathogen of foot-and-mouth disease is therefore the organic mold of the potato; the tubers of this plant are the original source of the virus.

The agent of foot-and-mouth disease is considered a filtering virus, because the lymph of the canker sores, filtered through porcelain, is still active; it has been shown elsewhere that this fact in no way demonstrates the existence of a virus containing only filtering elements; these are the zymospores of the cultures; these zymospores coexist there with other bacterial elements, but they pass through the filters alone because of their rounded shape and their lively Brownian motion; they are by no means invisible; they are easily seen at medium magnifications (1000 to 1200 D), because they are from 0.2 to 0.5 μm.

The cloudy or purulent lymph of canker sores contains (Fig. 14, pl. 276) all the characteristic elements of bacterial cultures of organic potato mold (Fig. 7, pl. 145).

Broth cultures are very easily obtained by inoculating a drop of this lymph, especially that of hoof sore, into glycosylated peptone veal broth, stored at a temperature of 18-24°. Gonidian forms of the pathogenic mold are also easily obtained by the following methods:

1. By depositing canker sore lymph over the entire surface of a sweetened carrot and potato (5% maltose, 5% glycose) and on the upper half of the surface of an agar tube.

The cultures are kept almost horizontal for twenty-four hours and then upright; the gonidial forms appear from the third to the eighth day; it is necessary to carry out cultures on these three media, because certain gonidial forms, *Mucor, Aspergillus*, for example, develop preferentially on carrot and potato.

2. By inoculating a drop of lymph in broth; when the culture has deposited or formed a film on the surface of the latter, the deposit or film is transferred to the previous solid media.

Often, the surface film transforms into fertile aerial mold on the very surface of the broth.

3. By inoculating the guinea pig with the lymph or the broth culture and culturing the pulp of the animal's organs on agar after death.

Bacterial culture and gonidial forms are obtained with barely smeared lymph from tongue ulcers; but the result is much more perfect using lymph from the hoof ulcer punctured with a sterile pipette before spontaneous opening; it is sufficient to perforate the thin wall of the ulcer with the tip of the pipette for it to fill spontaneously, without any suction, by the simple pressure of the liquid inside the ulcer; thus, up to 5 cubic centimeters of lymph are easily obtained in a single operation.

Isolation and study of gonidial forms of the pathogen.

In the first studies conducted on foot-and-mouth disease, I obtained several gonidial forms of the pathogen by direct transformation of a bacterial culture developed by inoculating the serosity of a tongue ulcer (animal from the La Villette slaughterhouse).

The forms obtained were:

1. A *Penicilium* form (Fig. 3 to 6, pl. 275 and fig. 1 to 7, pl. 276).

2. A gonidian form which appears intermediate between the *Penicilium, Spicaria,* and *Verticilium* forms (Fig. 2, pl. 275).

These two forms, identified later, were the first *Penicilium solani,* the second a form which I provisionally designate *Spicaria verticillata solani;* I, in fact, isolated this identical form by direct transformation on agar of the bacterial culture of potato rot (Fig. 7, pl. 148); it appears to have been observed by Karsten **(1)**.

From the same original culture that had already produced *Penicilium solani* and *Spicaria verticillata solani*, I subsequently obtained one of the most characteristic gonidial forms of the potato: *Fusarium solani*.

From the isolation of these characteristic gonidial forms, it resulted, without the slightest doubt, that the original culture obtained with the serosity of a canker sore on the tongue contained the elements of the organic mold of the potato.

In another study, carried out shortly after using serosity taken from five cows from the same contaminated stable (commune of Chagey, Doubs), I directly obtained, by culturing the serosity on carrots, the following gonidial forms:

1. A *Penicilium* form identical to the previous one.
2. A *Monosterigmata* form (Fig. 1, pl. 275).
3. A *Mucor* form not shown in the plates; this form is *Mucor solani.*

The first two forms identified were *Penicilium solani* and *Monosterigmatis solani*.

A third study on the serosity of a canker sore on the tongue (commune of Noirefontaine, Doubs) allowed the collection of:

1. *Penicilium solani* (Fig. 4 to 17, pl. 277).
2. A *Verticilium* (Fig. 8, 9, 10, pl. 276), the study of which showed the identity with the potato *Verticilium alboatrum* (*Verticilium solani)*, already obtained by direct transformation of the vaccine pulp (Fig. 3, 4, 5, pl. 263).

(1) *KARSTEN (Ann. der Landwirthch in K. P.).*

A fourth study was conducted on the serosity of canker sores from a dozen cows belonging to three stables located in three different communes: Sainte-Marie, Manbouhans, and Voujaucourt (Doubs). In the latter commune, the contaminated stable was located on an isolated farm 2 kilometers from homes and with only one road that was difficult for horse-drawn carriages and impassable for automobiles; the invasion of foot-and-mouth disease by contamination in this farm (Feuillebois farm) was unacceptable. With the collected serosity, the following was obtained for each stable:

A. Sainte-Marie stable, collected by culture of the serosity from a canker sore on the hoof:

1. *Penicilium solani.*
2. *Monosterigmatis solani.*

B. Manbouhans Stable; collected from the serosity of a hoof ulcer:

1. *Penicilium solani.*
2. *Monosterigmatis solani.*
3. *Verticilium alboatrum.*
4. *Fusarium solani.*

C. Stable of the Feuillebois farm (commune of Voujaucourt) collected from the serosity of udder ulcers:

1. *Penicilium solani.*
2. *Monosterigmatis solani.*

The serosity which, in each case, has already provided these gonidial forms, is still under study and will certainly provide new ones.

In summary:

1. In all the cases studied, without a single exception, the serum from the canker sores always provided at least two gonidial forms of foot-and-mouth disease, sometimes three, *and always, without a single exception, these gonidial forms were those of the organic mold of the potato.*

2. The molds reported are all those that were obtained; not a single different one was obtained that was not related to the potato; not a single form of the organic mold of beef was obtained.

3. The different gonidial forms obtained were:

A. *Penicilium solani.*

B. *Monosterigmatis solani.*
C. *Verticilium alboatrum* or *Verticilium solani.*
D. *Spicaria verticillata solani.*
E. *Mucor solani.*
F. *Fusarium solani.*

These results prove, beyond the slightest doubt, that the agent of foot-and-mouth disease is the organic mold of the potato.

Since an identical conclusion has already been drawn for the pathogenic agents of cowpox and smallpox, there must be a verifiable identity between the *Penicilium, Monosterigmata*, and *Verticilium alboatrum* forms of the three pathogenic molds. This is easy to verify on the plates and has already been reported in the study of cowpox and smallpox.

We will, moreover, obtain further confirmation of these different conclusions by studying bacterial cultures of foot-and-mouth disease.

Study of bacterial cultures of the pathogen of foot-and-mouth disease.

The lymph removed from a canker sore ready to open to the outside contains all the elements of the culture of the pathogen of foot-and-mouth disease; this lymph is therefore a complete culture; inoculated into broth preserved at 18-24°, it reproduces an identical culture; but, to confirm this identity, its daily development must be followed; certain bacterial forms, in fact, only appear in old cultures.

When examining a preparation of serosity recently removed from a canker sore (spread on a glass slide, desiccation and rapid heat fixation, dahlia staining), one is struck by the diversity of bacterial forms (Fig. 14, pl. 276; foot-and-mouth disease serosity, Noirefontaine): very large, medium, or small bacillary forms, coccobacilli, micrococci, etc.

This diversity, which is likely to confuse an uninitiated observer, does not correspond, as one might believe at first glance, to a bacterial mixture of different species; only the diverse forms of a single species exist in the serosity; these are the bacterial forms of potato rot or of the organic mold of this plant in old cultures (Fig. 1 to 8, pl. 145); these forms are found, in fact, in the rot of healthy potatoes heated for an hour or two at 55° (Fig. 7, pl. 145).

In this rot we observe:

1. A large bacillary form, 0.8 to 0.9 microns wide and 3.5 to 4 microns long on average, shorter when it segments.
2. An average form, 0.3 to 0.5 microns wide, of variable length.

3. A very small form, 0.15 to 0.2µm wide, of variable length.
4. Coccobacilli of various sizes.
5. Micrococci of various sizes.
6. Fine granules (zymospores) of 0.1µm to 0.5µm.
All of these identical elements are found in the serous secretion of mouth ulcers.

If the latter is inoculated into broth preserved at 18-24°, the development of bacillary forms identical to those which develop in a bacterial broth culture of the organic mold of the potato is observed (Fig. 11, 12, pl. 276). The thin bacillary elements (1 to 3µ) appear in large numbers in cultures aged eight days or more (Fig. 13). Cultures of both categories form, at least in the first generations, a veil which is initially bacterial and filamentous and which often reconstitutes a fertile aerial mold.

* * *

It follows from these observations that the study of all forms of the pathogenic agent of foot-and-mouth disease, bacterial forms or gonidial forms of molds, leads to the observation of their identity with those of the organic mold of the potato and with those of the pathogenic agents of vaccinia and smallpox already identified with the latter.

The organic mold of the potato is therefore the common original source of the pathogens of cowpox, smallpox, and bovine foot-and-mouth disease.

Let us add here that tetanus, whose pathogen has been determined to have the same original source, the organic mold of the potato, should have been included in the study of the three previous viruses.

Obtaining the same gonidial form, *Verticilium alboatrum* or *solani,* from the vaccine pulp and the serosity of bovine canker sores demonstrates the common origin of the two viruses that cause vaccine pustule in humans or canker sores in cattle. However, it is known that foot-and-mouth disease does not immunize the cow against the effects of the vaccine. It follows from this fact that, if the original source of the two viruses is the same, the gonidial form to which they each respectively refer is different.

Since the source of vaccinia is cowpox, it must be concluded that two different conditions can develop in a cow contaminated by two different gonidial forms of organic potato mold.

This fact is in agreement with other observations already made and presented previously: the various gonidial forms of the same organic mold, such as rat mold, do not cause the same lesions in the animals they infect.

Due to the differences in fermentative properties of the various gonidial forms of the same mold, it is very likely that inoculating an animal with the *Fusarium* form, for example, will not protect it against the bacterial effects of the *Verticilium, Monosterigma*, or *Aspergillus* forms, and vice versa.

The fermentative properties of a gonidial form exert such an influence that, despite the morphological identity of the *Penicilium* forms obtained with the vaccine, variola, or foot-and-mouth viruses, a difference is observed between the forms obtained by transformation of each of these viruses, which is manifested by the odor of the cultures. The *Penicilium* culture obtained by the transformation of the variola virus, for example, exhales a strong, characteristic odor (a strong musty odor, accompanied by another pleasant odor, difficult to define), which the *Penicilium* culture resulting from the transformation of the foot-and-mouth virus does not possess. Depending on their origin, *Penicilium* cultures, derived from different gonidial forms of the same species, also retain certain characteristics specific to them, but which they generally appear to lose after several generations and quite quickly.

We are therefore led to investigate the two gonidial forms of the organic mold of the potato which, vegetating in the cow's body, cause either cowpox or foot-and-mouth disease.

It has already been indicated above that the same research is being done for cowpox and smallpox.

This research, which is essentially that of the means of vaccinating cattle against foot-and-mouth disease, is already advanced; attempts are being made to obtain this vaccination, either by means of heated cultures of foot-and-mouth disease or potato pulp, or by means of heated potato juice itself.

This research will be the subject of a later publication.

* * *

Since the potato is the original source of the foot-and-mouth, cowpox, and smallpox viruses, it is easy to trace the various modes of contamination of individuals. Contamination can occur:

1. By the juice of the tubers soiling the hands that peel them.

2. By the molds that develop on the surface of potatoes stored in cellars (*Phytophtora* or *Mucor, Fusarium solani*, etc.).

3. By the liquid that oozes from rotten potatoes and by this very rot, either when the tubers are harvested (wet or brown rot in the soil) or during their storage in the cellar; the liquid and the rot are, as we saw above, a virulent bacterial culture.

4. By the liquids that flow from manure piles where potato peelings or unusable tubers are rotting and thrown into them.

5. By fermented foods, cooked potatoes, potato soup, that are several days old.
It is expected that cattle contract foot-and-mouth disease (and cowpox) mainly:

1. By inoculation with potato juice or mold spores contaminating hands that have peeled the tubers and that milk the cows without washing.

2. By crushing under the hooves and inclusion between the hooves of healthy or rotten tuber patches and juice; this commonly occurs during the tuber harvest; cattle used as draft animals frequently crush healthy or rotten tubers scattered on the ground in cultivated fields under their feet.

3. By inclusion of bacterial culture between the hooves trampling on the soil contaminated by manure or slurry containing this culture or by cooked potato soup, given as feed to pigs and spread on the ground.

Foot-and-mouth disease epidemics are increasing and becoming very frequent, especially at potato harvest time, which confirms that the two main ways of contaminating cattle must be: on the one hand, the crushing of rotten and healthy tubers under the hooves and, on the other hand, the soiling of human hands and shoes during the handling of rotten and healthy tubers. Humans subsequently contaminate the cow, either through their hands or through their shoes, which bring the bacterial culture into the stables.

In the transmission of foot-and-mouth disease, there is therefore no need to invoke contamination from one village to another; in the same stable, animals naturally transmit the disease, but they are also all subject to the same source of primitive contamination, the bacterial culture of the potato.

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From the careful examination to which I have devoted myself, it results that the appearance of the disease, in a stable, at least in the first one which is affected in a village, is certainly, and almost always, spontaneous. In isolated farms the development of foot-and-mouth disease by contagion is generally so incomprehensible that one has had to, to explain it, invoke the conservation of the virus from one year to the next, its transmission by insects, etc.

In reality, if the contamination of cattle (and formerly of man) by smallpox is so frequent, it is due to the ubiquity of the virus, either in the form of a bacterial culture or in

the form of fresh potato juice soiling human hands and animal hooves. It can be stated that the bacterial culture exists on all farms and in all manures; the opportunities for contamination are daily and throughout the year due to the continual handling of potatoes, the most common food of man and the daily food of pigs.

Disease prevention must therefore consist, first of all, in eliminating the virus from manure and soil by taking the greatest care in handling tubers: *by burning peelings, rotten tubers, and all potato debris* instead of throwing them on manure; by avoiding throwing the wash water from tubers prepared for food on the ground and on manure.

Careful hand washing after peeling or handling tubers, especially those stored in cellars and covered with the various gonidial forms of the potato, is an essential precaution.

It is advisable to avoid using cows for harvesting tubers; it is also necessary to wash and brush the hooves of horses used for harvesting and human shoes before entering the stables.

It seems necessary to isolate pig stables and prevent the flow outside of liquids always soiled by rotten potato debris.

But this set of precautions is very complicated and it is unlikely that they will be taken properly on farms. It seems certain that the most effective way to avoid foot-and mouth disease is preventive vaccination practiced in young animals.

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DISEASES CAUSED BY PATHOGENS CONSIDERED TO BE PROTOZOA:

SPIRILLAS, HEMATOZOA, AMOEBAS, TRYPANOSOMES, LEISHMANIA, PYROPLASMA, etc.

NATURE AND ORIGIN OF THESE PATHOGENS

A whole category of pathogens have been classified as animal parasites. The main ones are:

1. Spirochetes, spirilla or *Treponema*, notably those of syphilis, icterohemorrhagic spirochetosis, relapsing fever, etc.
2. Trypanosomes, notably *Trypanosoma gambiense*.
3. The hematozoan of malaria.
4. *Pyroplasma*, parasites of pyroplasmosis.
5. *Leishmania,* parasites of Kala Azar.
6. The dysenteric amoeba.

Let us examine their nature in turn:

1. Spirochetes and spirilla are not protozoa; they are spiral or wavy filaments that develop on the mycelial branches of molds.

The demonstration further on of the nature of *Treponema pallidum*, a spiral branch or appendage of the mycelium of the syphilitic mold, shows that spirochetes or spirilla are not animal parasites possessing individuality.

The isolation, explained further on, of the pathogenic agent of icterohemorrhagic spirochetosis, in the *Aspergillus* and *Penicilium* forms of a mold, completes this demonstration. The spirochete, considered as the agent of this disease, is a spiral filament detached from the mycelium of the pathogenic mold, which is the *Aspergillus* form of the rat's organic mold.

These spiral filaments, which have been taken for individualized animal parasites, develop as well on the mycelium of the *Mucor, Aspergillus*, and *Monosterigmata* forms of organic molds of plants as on those of animals. It has been demonstrated, in several places of this book, that all the membranes of animal and plant organisms are made up

of a very tight web of these spiral filaments, developed laterally and at the ends of mycelial branches of organic mold and spread out on a single plane. Everywhere, in all points of a plant organism or an animal organism, we find these spirillary forms.

It is therefore inevitable that, in an ulceration of the mouth, for example, we always find, alongside the fusiform bacillus; we find it just as well in healthy subcutaneous connective tissue.

Spirillae are therefore not animal parasites; they are spiral mycelial filaments, detached from the mycelium of a mold; it does not matter whether the mold belongs to an animal or a plant from which it emanates; once removed from the organism, it becomes a common mold with vegetative properties similar to organized mold.

These facts explain why it has never been possible to obtain cultures of spirochetes, an achievement as impossible as that of reproducing a fruit without cultivating the tree that bears it.

Spirochetes, notably *Treponema pallidum*, are driven by a double movement of rotation and progression, often accompanied by a rocking movement. This movement is passive and not active; it is determined by one or more zymospores attached to the spirochete; these, driven by Brownian motion and a lively dance and progression movement, carry the element with them and are the cause of its progression and rocking movements; as for the rotational movement, it results mainly from the spiral shape of the element;

being driven forward by the zymospore, the resistance of the liquid necessarily imparts a rotational movement to it.

It is known that certain spirillae with long undulations only present their undulating appearance when they are in motion and that this undulation disappears as soon as the movement ceases. These are simple straight mycelial filaments which are set in motion, accompanied by undulations, by zymospores which attach themselves to them and which carry them along by giving them their oscillating movements; these undulations are identical to those caused by a hand waving the end of a rope in water; they are passive movements communicated by the movements of the zymospores.

2. Trypanosomes are vegetative elements of molds analogous to the fixed cells of the connective tissue of animals.

It is established later, during the study of the pathogenic agent of sleeping sickness, that trypanosomes are mycelial dilations of molds analogous to the fixed cells of the connective tissue and not individualized animal parasites; this work provides photographs of microscopic preparations showing, taken on the spot, the mode of formation of *Trypanosoma gambiense*, in connection with the germinative mass which gives rise to it; normal elements of the form of trypanosomes always exist in considerable numbers in the liver, spleen, nerve centers, etc., of all animals; we can give as an example those which are shown in Plate 11 and which are constant elements of the spleen of the mouse.

Being located at the end of a very fine and flexible mycelial filament, these formations appear to be independent organisms when they are detached and launched into the circulation as in the rat and the mouse. If a zymospore animated by Brownian movement comes to attach itself to the flexible and fine stalk of the formation, and especially to its end, it determines an undulatory movement and carries the entire formation with it; the latter consequently appears to be animated by an active autonomous movement which, in reality, is only passive, *all the activity being localized to the zymospore which is foreign to the formation and has fixed itself against its stalk as it fixes itself to all the other elements in suspension in the medium;* the movement imparted here has the same cause as the bacillary movements due solely to passive movements provoked by the zymospores.

3. The malaria hematozoan has no individuality and is not a protozoan; it is an element of the pathogenic mold that is the agent of malaria (spore, evolving germinal mass, or fruiting body).

It is demonstrated later, in the study of malaria, that Laveran's hematozoan is either a spore of intramycelial origin, or a stage in the evolution of the germinal mass, or even a fruiting body of the pathogenic mold.

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These various elements are formed in in vitro cultures of the mold; the complicated cycle of the evolution of the hematozoan is imaginary; this cycle of evolution is that of all molds developing in liquid environments; there is neither a sexual nor an asexual cycle, nor male nor female gametes; the evolution of the pathogenic agent has no need to take place in an intermediate host, the mosquito, the disease being transmitted, like any other, by inoculation of any bacterial elements, spores and zymospores for example; it is obvious that the disease can be inoculated by a mosquito which successively bites a sick person and a healthy person. But the identification of the pathogenic mold of malaria establishes that there is direct or indirect contamination by the organic mold of rice, and that an individual can contract malaria in a place where there are no infected individuals, but where there are rice fields. If the inoculation is indirect, it is likely that the biting mosquito becomes infected by landing on the mud of the rice fields or on the rotting rice straw.

4. Dysenteric amoeba.

I have not had the opportunity to study amoebic dysentery; but, according to the studies carried out and the known figures of the dysenteric amoeba, it can be affirmed that it is not an amoeba, but a germinative mass of a mold that is easy to isolate.

It is formed by an agglomeration of bacterial elements, notably spores. In the figures given, we see germinative masses in all stages of development, from the mass formed only by the agglomeration of three or four spores. In the figures representing amoebic cysts, we notice, alongside round spores, bacillary forms. These are elements which, by their agglomeration, form the germinative masses in all cultures.

The fact that, in an epidemic localized to a well-circumscribed region, some of the patients suffer from amoebic dysentery, others from bacillary dysentery (*Shiga* and *Flexner)*, and that in a certain number of others, both the amoeba and the dysentery bacillus are found, suggests that the amoeba is most likely the germinative mass of the bacterial culture of the *Shiga* and *Flexner* species, that is, species of the genus *Brassica,* which develops in the human intestine.

5. Piroplasma.

The parasites of piroplasmosis and those of leishmaniasis are also mold elements in various stages of their development; they are either germinative masses or terminal mycelial formations, like trypanosomes; the known figures of piroplasms and the parasites of Kala-Azar leave no doubt about their nature; these parasites have no individuality and are the elements of a pathogenic mold.

* * *

In summary, the pathogens currently considered protozoa are of a very different nature; they are not animal parasites, but elements of a pathogenic mold whose original source is a plant or animal organism. Their development does not involve the complicated cycle described for the malarial hematozoa and the dysenteric amoeba. This development occurs according to the cycle indicated for bacterial cultures and molds in the mycelial state with the four phases of agglomeration, fusion into germinative masses, emission of mycelial filaments, segmentation of the mycelium into bacterial elements, and emission of fruiting bodies (spores and zymospores). *This development always occurs entirely in infected organisms;* it does not require an intermediate host, such as an insect. When disease transmission occurs by a biting insect, the latter serves only as a carrier of the virus.

Biting insects do not only feed on the blood they take from bitten animals; mosquitoes, for example, suck the sweat from living plants and feed on it. It is easy to convince oneself of this on the banks of a river heavily populated with mosquitoes; one sees them land on leaves and apply their perforating apparatus to their surface to sting them and pump out the sweat; if a mosquito is approached carefully and examined with a magnifying glass in this position, there is no doubt about the operation it is performing.

Biting insects also land on decomposing matter, into which they engage their perforating apparatus; they carry away the bacterial cultures they contain; a bacterial culture provides food for a mosquito as well as blood for an animal.

A biting insect therefore directly inoculates humans with the reproductive elements (spores, zymospores, and others) of the organic mold of animals or plants from which they have previously pumped blood or juice, or of the bacterial cultures they ingest by landing on decomposing plant or animal matter.

SYPHILIS

The pathogen of syphilis is a mold that vegetates in the initial chancre in the gonidial form *Monosterigmata,* and which gives rise to a multitude of small, very fine branches rolled into a spiral which, when detached, constitute Schaudinn's spirilla (*Spirochæta pallida* or *Treponema pallidum*); the original source is the organic mold of monkeys.

Gonidial forms of the syphilitic mold.

Monosterigmata form

I isolated the syphilitic mold, for the first time, in the following way:

The scab of a chancre being torn off, and the exposed surface sponged with sterile cotton until no more blood flows, the serosity appearing on the chancre is taken with a pipette; this liquid is inoculated into glycosylated peptone veal broth (ordinary broth) *maintained at laboratory temperature* (18-24°). From the first culture that I made in this way, there appeared, after eight days, floating in the broth, a small mycelial colony the size of a millet grain which, developing, became as big as a pea; the colony having been fragmented to transfer part of it to agar and to make preparations, a large number of new ones developed in the broth three or four days later; the fragmentation of the first had released the elements which formed the following ones.

This mold, spread on a slide and stained with dahlia, immediately revealed that it was indeed the pathogen of syphilis because its mycelial branches gave rise to a multitude of fine filaments coiled in a spiral which, obviously, were *Treponema pallidum* (Figs. 4 and 6, pl. 283).

Transferred to agar, the mold develops, taking on a mouse-grey tint, and forming a large, very prominent, irregular, mammillary eminence. It often forms, on the surface of the medium, a bacterial culture which develops and flows towards the lower part in the form of a thick, cream-like substance of reddish-brown color.

Examined under a microscope, the mold has a characteristic appearance that makes it easily recognizable (Fig. 1, 2, 3, 5, pl. 283 and fig. 1, pl. 284); it takes the gonidian form *Monosterigmata.* Like the submerged mold, the gray aerial mold has a multitude of fine filaments coiled in a spiral on its branches (Fig. 2, pl. 284).

Transferred to carrots, the mold spreads on the surface, developing a bacterial culture by segmenting its branches; the elements of this culture are of two forms, a straight bacillary form and another form, very characteristic of syphilis, consisting of an oval

element, rarely spherical, not stainable by the dahlia, bearing at one or both ends a straight cylindrical bacillary body, of variable length, strongly stainable blue by the dahlia (Fig. 6, pl. 283. Upper right corner). A few rare oval elements do not bear a bacillary body; one then notices a blue-colored point at each end. It is easy to see, by examining unsegmented mycelial filaments, that this characteristic bacillary form results from the very shape of the mycelial filaments and the location of the segmentation points.

The scab of the same canker, whose serosity gave rise to this culture, was fragmented and its pieces applied to the surface of a carrot, the outer or epidermal side against it. A mold of the form *Mucor corymbifer* developed on the surface of one of the scabs. Unfortunately, this mold liquefied before being transferred to other media and was thus lost for study at that time. Later, it was again obtained by indirect transformation of the previous form *Monosterigmata* (inoculation into the guinea pig and organ culture).

The *Monosterigmata* form of the syphilitic mold occurs in a second type, the culture of which, milky white in color, has the form of irregular manmelons strongly protruding at each point of inoculation. This form was obtained by transferring the previous gray form to broth to transform it into submerged colonies; these formed a white veil which emitted aerial fruiting bodies; transferred to agar, the mold takes on a milky white color. It still has the *Monosterigmata* form, but its fruiting bodies differ notably from those of the proceding type. The gonidiophores or sterigmata no longer present the characteristic swelling of the other type; the spores are smaller, spherical or oval, irregular, and have a thick, non-basophilic external membrane (Fig. 3 to 7, pl. 284).

Penicilium Form

The Penicilium form of the syphilitic mold was obtained directly from the transformation of the gonidial form Monosterigmata. A grey monosterigmata culture evolved spontaneously, after three to four months, into the Penicilium form (Figs. 1 to 8, pl. 285).

I also obtained it indirectly, by inoculating the guinea pig with the spores of the *Monosterigmata* form and culturing the pulp of its organs (Figs. 4, 5, 6, 8, 9, 10, pl. 286 and fig. 2, pl. 287). Three molds of the gonidial forms *Monosterigmata, Mucor corymbifer*, and *Penicilium* were thus obtained directly from the serosity of the same chancre.

The search for the *Penicilium* form of the syphilitic mold must be accompanied by careful control with regard to the guinea pig organic mold, because certain types of the latter, that of Fig. 1, Plate 287, for example, can be confused with it.

The other gonidial forms of the syphilitic mold that follow were all obtained indirectly.

Mucor corymbifer form

It has just been stated that it was obtained directly by culture of chancre scabs. It was also obtained by culture on agar of the organ pulp of a guinea pig inoculated with the spores of the gray *Monosterigmata* form (Figs. 1, 2, 3, Plate 286).

Organ culture from this animal also produced the *Penicilium* form (Figs. 4, 5, 6, 8, 9, 10, pl. 286) and the *Aspergillus minimus* form (Fig. 7, pl. 286).

Sterigmatocystis form (Fig. 3, pl. 287).
Aspergillus form (Fig. 8, pl. 287).
Aspergillus minimus form (Figs. 5, 6, 7, pl. 287).

These last three forms and, in addition, the *Penicilium* (Fig. 2, pl. 287) and *Grey Monosterigmata* (Fig. 4, pl. 287) forms grew in cultures of the organ pulp of a guinea pig inoculated with the spores of the *Grey Monosterigmata* form.

The *Aspergillus minimus* form is a *Sterigmatocystis* or Penicilium incompletely developed; its divaricate appearance is characteristic.

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In summary, the following gonidial forms were obtained from a single syphilitic chancre:
1. *Monosterigmata*, in two different types, gray and white.
2. *Mucor corymbifer*.
3. *Sterigmatocystis.*
4. *Aspergillus.*
5. *Aspergillus minimus.*
6. *Penicilium.*

Identification of the syphilitic mold.

Gonidial forms 1, 2, 5, 6 have a characteristic appearance that will allow for sure identification.

From the examination of these gonidial forms of the syphilitic mold, it appears, firstly, that they have no common link with human organic mold; the pathogenic agent of syphilis is therefore heterogeneous.

I found no connection between the gonidial forms of syphilitic mold and those of the organic mold of the most common plants or animals that serve as food for Europeans or that are in frequent contact with them. However, I did note the identity of the gonidial forms *Mucor corymbifer, Penicilium*, and *Monosterigmata* with those of the organic mold of the macaque monkey (Pl. 288, 289, and 289 bis).

The organic mold of this animal, like the syphilitic mold, has a *Mucor corymbifer* form, an exceptional fact, because it is the only animal in which I have encountered it.

If this identification were correct, it should be possible to find, by transformation of the gonidial forms of the macaque's organic mold, the *Monosterigmata* form characteristic of syphilis with the type of its gray culture or that of its white culture. In order to obtain it, the *Mucor corymbifer* form of the monkey's organic mold was inoculated into the guinea pig; the culture of the animal's organs gave rise to both the *Penicilium* form and the white *Monosterigmata* form characteristic of syphilis; obtaining this latter form constitutes, in itself, confirmation of the origin of the syphilitic mold: it is indeed the macaque's organic mold.

Following this observation, the existence of spirochetes was sought in cultures of the pulp of organs of guinea pigs inoculated with the *Mucor corymbifer* of the macaque. In these cultures, a matter of whitish creamy consistency developed below the inoculated area, consisting of a culture of various bacterial elements; the bacillary elements it contains are identical to those observed in cultures of the same genus developed on the pulp of organs of guinea pigs inoculated with the syphilitic mold that develops on them, and the preparations contain a large quantity of these elements. They no longer have the same shape as those represented in figure 6, plate 283. They are straight bacilli, completely basophilic, of variable length according to their degree of segmentation.

This creamy material, being inoculated into broth preserved at 1800, behaved exactly like the serosity of human syphilitic chancre. After four days, small floating, spherical mycelial colonies developed, the filaments of which were covered with a multitude of spirochetes. These colonies evolved directly into the *Penicilium* form instead of giving the *Monosterigmata* form.

This study, which is continuing, will probably soon establish the identity of all the gonidial forms of the organic mold of the monkey with those of syphilis, since verification has already been made for three of the most characteristic.

The macaque's organic mold has a second, very characteristic gonidial form, *Mucor* (Fig. 4, 5, pl. 288). It is likely that it can be obtained from the syphilitic mold in cultures of the organs of inoculated animals.

It is currently believed that animals do not contract syphilis because the virus inoculated by intradermal injection or scarification does not cause the development of chancre. This is a mistake. The guinea pig, for example, contracts syphilis by subcutaneous inoculation and dies, although no chancre ulceration forms at the site of inoculation:

about eight to ten days after inoculation, the animal's blood contains a multitude of spirilla. *The syphilitic mold has vegetated in all parts of the body, because its gonidial forms develop on the pulp of all the organs: lung, liver, spleen,* etc. All animals inoculated with syphilis contract it.

The identification research carried out above has led to the conclusion that the original source of syphilis is the specific organic mold of various species of monkeys, notably that of the macaque monkey.

This explains why this animal cannot contract this disease, the pathogenic mold of the latter being the one that constitutes its organism. *It seems obvious, a priori, that the organic molds of other species of monkeys, and even those of higher monkeys, must constitute species of syphilis similar to the previous one.*

I had at my disposal two cercopithecines and a chimpanzee and was able to isolate the principal gonidian forms *Penicilium, Sterigmatocystis, Aspergillus, Mucor, Monosterigmata*, etc., from the organic mold of these two species of monkeys.

From the Cercopithecus I isolated the white *Monosterigmata* form, perfectly identical to that of *Syphilis*, and forming an innumerable quantity of spirillae like these. *There therefore exists a form of Cercopithecus syphilis*.

From the chimpanzee, I isolated a *gray Monosterigmata* form. The macroscopic appearance of the culture is similar to that of the *gray Monosterigmata* form of syphilis; the mold has longer hairs, is darker gray, and forms a slightly less prominent eminence than the culture of the *Monosterigmata* form of common syphilis. However, upon microscopic examination, the gonidiophores are found to have a shape similar to those of the *Monosterigmata* form of syphilis (Fig. 1, pl. 283). *Therefore, there probably exists a form of chimpanzee syphilis.*

The gonidial forms of the organic mold of the cercopithecus and the chimpanzee are photographed; as they cannot be produced in this work, they will be included in another publication.

Currently, three forms of syphilitic mold have been isolated; I say three forms, since the monkeys that are the original source of these diseases are specifically different. It is likely that the most common form of syphilis is that of the macaque. But it is certain that the natives of regions where cercopithecines and chimpanzees exist are contaminated by these animals with which they often live in promiscuity; it is not uncommon for travelers to tropical regions to bring back photographs showing us this promiscuity **(1)**. It is therefore very likely that this syphilis must have spread among Europeans.

(1) *NEVEU LEMAIRE, Two Travels in West Africa, Pl. 6; woman carrying a chimpanzee; Geographical Society, 1920.*

I have not yet investigated whether the serum of animals inoculated with the syphilis of the cercopithecus and the chimpanzee gives a positive Wassermann reaction. This is a question of the utmost importance for the treatment of syphilis and will soon be elucidated.

I have studied the action of salvarsan on the syphilitic mold. This study is explained later in connection with sporothricosis, a form of syphilis.

Knowing the original source of syphilis, we arrive at the knowledge of the origin of this disease for the inhabitants of temperate regions.

It seems obvious, a priori, that syphilis was first transmitted, originally and at all times, to the inhabitants of the regions where monkeys live; this transmission can take place by biting insects, flies, mosquitoes, fleas, lice, etc., or by the ingestion of monkey meat, or by direct contact, the cohabitation of man and monkey being frequent in tropical regions. It is known that syphilis is extremely common among the natives in certain regions.

It is therefore through the natives of the regions that are the natural habitat of monkeys that syphilis was transmitted and spread to the inhabitants of regions deprived of these animals.

Until now, many have considered monkeys to be inferior stages in the evolution of the human organism. By applying this hypothesis, monkeys have been used as experimental subjects capable of replacing man, believing that their organic reactions should be as close as possible to his own; it has been believed, for example, that they would constitute an animal of choice for the study of syphilis; this is an error explained by knowledge of the original source of this disease; the original source of the syphilitic mold being the organic mold of the macaque or other monkeys, it is understood that inoculating the latter with its own organic mold does not give it typical syphilis.

SPOROTHRICOSIS

Sporothricosis was long confused with syphilis, which means that it clinically presents its characteristics. I have compared it here to syphilis because the transformation study I conducted on *Sporothricum Beurmani* and *Gougeroti* demonstrated that these two molds are identical and are a gonidial form of the syphilitic mold.

I carried out this study using cultures of *Sporothricum Beurmani, Sporothricum Gougeroti*, and *Sporothricum Schenki*, which Dr. Gougerot kindly made available to me. From these cultures, I obtained the gonidial form *Penicilium*:

1. By spontaneous transformation; I only obtained it for *Sporothricum Beurmani* and *Sporothricum Schenki*.

Having transplanted the cultures of *Sporothricum Beurmani* onto carrots, I observed that they managed to form erect aerial filaments, preferably in the driest regions of the culture medium (at the top of the carrot). Transplanting these aerial filaments several times onto previously prepared carrots with little moisture, I obtained a completely aerial mold, mouse-grey in color, with hairs 3 to 4 millimeters high. One of the transplants of this mold spontaneously transformed, at the periphery of a colony, into a green mold of the *Penicillium* form shown in Figures 5, 7, and 8, Plate 290.

I obtained the *Penicilium* form of *Sporothricum Schenki* by the same process; the first culture with aerial filaments, when subcultured on agar, produced a yellowish-cream mold of the form shown in Figure 1, Plate 291. This is a first stage in the evolution of *Sporothricum* toward the *Penicilium* form. One of the subcultures on agar of this mold spontaneously transformed into a green *Penicilium* culture (Fig. 2, Plate 291).

2. By indirect transformation; the original cultures of *Sporothricum Beurmani* and *Sporothricum Gougeroti* were inoculated into guinea pigs. After death, the pulp of the lung, liver, and spleen of the animals, cultured on agar, gave rise to numerous colonies of *Penicilium*.

Those which developed on the cultures of the organs of the animal inoculated with *Sporothricum Beurmani* (Fig. 4 and 6, pl. 290) were recognized as identical to that which had been obtained by direct transformation of the original mold.

On the other hand, the three cultures of *Penicilium* derived from *Sporothricum, Schenki, Beurmani* and *Gougeroti* (Fig. 3, 4, 5, 6, pl. 291) were recognized as identical; these three *Sporothricum* therefore do not represent species, but only varieties or diverse aspects of a single species of *Sporothricum.*

The figures given here of the three *Peniciliums* obtained are few in number, because at the time they were made and photographed, I had difficulty obtaining well-developed cultures and suitable preparations. It was only later that, comparing these more perfect culture forms of these *Peniciliums* with that of the syphilitic mold, I established their identity; this cannot be in the slightest doubt, and it is possible to verify it even with the aid of the figures contained in the plates alone.

On the other hand, there is a striking identity between certain types of the *Penicilium* form of the three varieties of *Sporothricum* and those of the *Penicilium* form of the organic mold of the macaque monkey.

From this identity, it follows that *Sporothricosis* is indeed a manifestation of syphilis, but that it takes on a particular aspect because the pathogenic mold vegetates, not in its usual monosterigmatized gonidial type, but in the *Sporothricum* type.

Currently, new research aims to achieve the transformation of *Sporothricum* into one of the characteristic gonidial forms of the syphilis agent, the *Monosterigmatized* form or the *Mucor corymbifer* form, for example, in order to determine from which species of monkey sporothricosis originates.

* * *

From the preceding facts, it is useful to draw out an observation of great importance:

If the serum of syphilitic patients with the *Sporothricum* gonidal form does not give the Wassermann reaction (whatever its nature) that the serum of syphilitic patients with the *Monosterigmata* gonidal form gives, this fact means:

That it is impossible to reliably diagnose, by a single complement fixation reaction, a disease that can be caused by several different gonidial forms of a pathogenic mold and even by specifically different molds; the serum corresponding to each disease, reacting only with the gonidial form or with the species of mold that determines it, will not give the positive fixation reaction if one uses as antigen a product from another gonidial form of the same species and, even more so, from another species.

If there are different forms of syphilis caused by the forms *Mucor, Monosterigmata, Aspergillus, Sterigmatocystis, Sporothricum,* etc., and one uses only as antigen the most common *Monosterigmata* form or a product that contains its elements, one will therefore obtain a negative Wassermann **r**eaction with the serum of patients affected by the gonidial forms *Sporothricum, Sterigmatocystis, Mucor,* etc.

The difficulty is complicated by the existence of syphilitic molds of different origins and originating from various species of monkeys. Since the possibility of carrying out a suitable or accurate fixation reaction for each disease is linked to the knowledge and, consequently, to the isolation of the gonidial form that determines it and to the knowledge of the original source of the pathogenic mold to which this gonidial form belongs, *it follows that the diagnosis will be more certain if it is carried out by the identification of the isolated mold.*

The specific determination of the pathogenic mold and its original source is relatively easy; it is isolated without difficulty by inoculating the guinea pig with a portion of tissue or liquid containing its elements, and the pulp of the animal's organs is cultured on agar. However, determining the gonidial form in vegetation is difficult. It can only be

done with certainty by direct examination of a portion of tissue in which the pathogenic mold is growing.

Direct microscopic examination of tissues affected by the syphilitic lesion allows for the recognition of the mold's mycelium, its fruiting bodies, or certain characteristic parts, such as the spirillary filaments formed on the mycelium and which constitute *Treponema pallidum*. The following conclusions can be drawn from the established facts:

1. The observed presence of *Treponema* proves the presence of the *Monosterigmata* form of the syphilitic mold.

2. The absence of *Treponema* and the absence of a positive Wassermann reaction do not prove the absence of syphilis. Syphilis exists in other gonidial forms than that which gives rise to *Treponema* and which determines a positive Wassermann reaction; there are various species of syphilitic molds with different original sources.

3. The diagnosis of syphilis must include the isolation and identification of the pathogenic mold. It must also include the search by microscopic examination for the gonidial form of the pathogenic mold which vegetates in the lesions.

4. The disappearance of the spirillae from a lesion does not mean the death of the syphilitic mold; the disappearance of *Treponema* can identify as a cause a change in gonidial form.

* * *

In the treatment of syphilis, the disappearance of *Treponema* does not therefore signify a cure. This can only be proven by the impossibility of obtaining one of the gonidial forms of syphilis by culturing the blood or other elements of the contaminated organism.

I studied the action of the drug currently reputed to be the most active against syphilis, salvarsan (neo-salvarsan). The monosterigmata mold grows on the surface of broth or agar containing a proportion of salvarsan two to three times greater than that contained in the blood after intravenous injection of 0.50 grams of this body. Beyond this dose, the development of the mold slows down, then ceases completely with a dose four to five times higher than the concentration of the drug in the blood.

From this experiment I do not deduce that the action of the drug in the organism is the same as in in vitro culture; indeed, the vegetative activity of the mold may be weaker in the human organism than in in vitro culture; in this case the drug would exert its action in the organism at a lower dose than in in vitro culture. But this is a hypothesis that is not supported by any known fact, and perhaps the opposite exists.

I note here that the study of the action of the drug, by observing the movements of the spirillae, cannot provide any serious information in such a study; the movements of the spirillae are passive and caused by the zymospores which attach themselves to them; the movements of the zymospores are not affected by the action of drugs used in enormous doses; one can observe the movements of zymospores in almost pure bleach; this phenomenon is due to the fact that Brownian movements have no relation to the vitality of the element and only to its dimensions; mineral and inert granulations possess Brownian movement.

ICTEROHEMORRHAGIC SPIROCHAETOSIS

It is accepted that the pathogen of this disease is a spirochete observed in the blood of patients. As in the case of syphilis, this spirochete is neither a protozoan nor the actual pathogen of the disease. It is a mold growing in the blood of patients in the form of *Aspergillus*, whose mycelium gives rise to a multitude of spiral filaments which, when detached, constitute the elements (spirochetes) that have been considered individual pathogenic elements.

I was able to study this disease thanks to the kindness of Professor Auguste Pettit of the Pasteur Institute, who kindly provided me with inoculated guinea pigs.

The pathogenic mold is obtained by cultivating on agar the pulp of the lung, liver, and spleen of animals that have died naturally or killed five to six days after inoculation; the cultures are covered after three or four days with blue-green, sometimes green, colonies of an *Aspergillus* form and olive-green colonies of a *Penicilium* form; at first, pure colonies of *Aspergillus* are obtained (Figs. 1, 2, 3, pl. 292), but these, when replanted, almost always give rise, by transformation, to the *Penicilium* form, which is thus found mixed therein. On the other hand, the *Penicilium* form, once isolated, remains pure (Figs. 4 to 9, pl. 292).

This *Penicilium* form is, without a doubt, that of the organic mold of the rat.

This explains the fact that the rat, which is known to be the carrier and source of the virus, does not suffer from it at all; the virus is its organic mold.

Preparations of the *Aspergillus* form intended for microscopic examination are covered with an innumerable quantity of very fine spirillae or their fragments (spirochetes). These spirillae are fragile and fragment during handling; they are barely colored by dahlia, because they are quite difficult to distinguish with this stain; it is therefore necessary, to see them clearly, to use another coloring process. But, even in prepara-

tions colored strongly by dahlia, one can clearly distinguish spirillae emanating directly from the mycelium.

In addition to the *Aspergillus* and *Penicilium* forms, I isolated the *Mucor* form of the pathogenic mold; it is identical to the *Mucor* form of the rat's organic mold, a fact that completes the identification established above.

The *Aspergillus* form of the pathogenic mold, inoculated into guinea pigs, causes death in ten or fifteen days with the characteristic lesions of icterohemorrhagic spirochetosis.

The inoculated guinea pigs that Mr. Pettit placed at my disposal presented, in addition to liver lesions, a large spleen that, in culture, yielded abundant colonies of *Aspergillus*; in the guinea pigs inoculated with *Aspergillus* spores, there was also, in general, such considerable hypertrophy of the spleen that, in one case, it presented two large bursting holes, its volume having reached that of a large walnut. The liver and spleen are often stuffed with tubercles.

The *Penicilium* form of the mold is as virulent as the previous one and kills the guinea pig in about ten days; but it does not cause the same lesions as the *Aspergillus* form; the liver is large, cirrhotic, and speckled with yellow; there is some jaundice and internal congestive lesions of the lung.
These notions, which seem simple at first glance, are less so when we carry out a check.

Indeed, the organic mold of the rat, which has just been indicated as being the source of the icterohemorrhagic spirochetosis virus, also appears, according to the previous study, to be the source of the plague virus. How can we explain that the organic mold of the rat can be the source of the viruses of both these diseases?

It has already been shown in the study of plague that bacterial cultures of rat organic mold (liver and brain cultures) kill guinea pigs in three or four weeks without producing any characteristic lesions. These cultures do not have the same virulence and do not produce the same lesions as plague cultures or the blood of animals suffering from spirochetosis.

It must therefore be concluded here that the character of the disease depends on the gonidial form of the contaminating mold and that several gonidial forms of the same mold, that is, having a common original source, produce several different diseases.

MALARIA

The pathogen of malaria, discovered by Laveran, is thought to be a small spherical or oval body 1 to 2 microns in diameter, which, upon enlarging, takes on the appearance of a ring; when fully developed, it reaches a size of 10 to 15 microns (or more for certain amoeba-like forms). It is considered a protozoan (*Laverania malariae, Plasmodium malariae, Hemamoeba malariae*).

The pathogenic agent of malaria is not a hematozoa; it is a mold easily isolated from the blood; the different forms of Laveran's hematozoa are spores or germinal masses of this evolving mold; these elements are not the most characteristic of the pathogenic mold and the only ones found in the blood of malaria patients; there is another which is abundant there and is the most characteristic of the mold: it is the gonidia. This conclusion is rigorously established by the following facts and can leave no room for uncertainty.

I studied the pathogenic agent in various cases of malaria; it is during a febrile attack that the pathogenic mold must be looked for in the blood. I owe it to the kindness of Doctor Valleteau de Mouillac, of Cherbourg, to have been able to conduct a complete study under excellent conditions. While caring for a malaria patient (infected in Macedonia), suffering from violent attacks, he took a sample of venous blood at the peak of one of them (temperature rising rapidly to around 41°) and sent it to me. Here are the facts that I was able to observe in the study of the blood:

Examination of the blood of a malaria patient during an attack.

It is venous blood, taken from a vein in the arm, at the most acute moment of the crisis, which was the subject of the following observations: for the examination, it is preferable that the blood be citrated; if it is not, we examine, on the one hand, preparations of the natural serum and the pellet obtained by centrifugation and, on the other hand, the clot crushed with a little salt water. The liquid is spread on a slide, dried and quickly fixed by heat, then the preparation is stained with dahlia and washed with hot water to remove the excess dye.

Figures 1 to 5 of Plate 293 are photographs of preparations made under these conditions. The blood contains all the elements of the malaria mold; these elements are:

1. The mycelium of the mold.

It is observed either in the form of isolated filaments in which the mycelial segmentation is clearly apparent (Fig. 4, pl. 293), or in the form of groups of filaments (Fig. 3), or in the form of large, long mycelial trunks emitting ramifications (Fig. 1), or even in the form of shorter fragments (Fig. 2). These mycelial filaments carry the sterigmata which give rise to the gonidia. These sterigmata exist in figures 1, 2, 3, 5. They often have the characteristic form they present in in vitro cultures of the malaria mold obtained in the gonidia form itself which vegetates in the blood.

2. The gonidia.

These are the elements which best characterize the malaria mold in the blood; they are identical to the gonidia of the mold cultivated in vitro; The largest mature gonidia are 4 to 6 μm wide and 5 to 8 μm long on average; there are smaller ones of all sizes, in the process of development; they have an oval shape, slightly pointed at the free pole (Fig. 2, pl. 293); the other pole sometimes bears a part of its pedicle which has remained adherent there or the trace of its insertion on the mycelium if it was sessile (truncated pole). The gonidia are born either at the end of a mycelial filament (Fig. 2 and 3), or at the end of sterigmata formed on the mycelial branches (Fig. 1, 3, 5), or on these directly, on which they are then sessile. Figures 2 and 5 show a mycelial branch bearing sessile gonidia.

3. Non-basophilic refractile spores of 1 to 2 μ.

In the mature gonidia, round refractile spores with a diameter of about one and a half microns are formed; they can be seen in the gonidia, still equipped with a vestige of its pedicle, which is located at the top of Figure 2. These spores, once released, constitute the elements considered until now as the first stage of the development of the hematozoan.

This origin of the round refractile spores is not the only one: they also form at the ends of thin mycelial branches and inside branches of a certain size (intramycelial spores). These refractile spores released into the blood are observed in figures 1, 2, 3 and especially in figure 4 (lower half) where they are clearly isolated. These spores are almost completely devoid of basophilic protoplasm and are very poorly stained by the dahlia.

Subsequently, basophilic zymospores attach themselves to these spores and fuse their protoplasm; when scanning the preparations, one can clearly see spores carrying a

single, sometimes two, basophilic zymospores of 0.2 to 0.3 μm in diameter, generally with the same diameter at the ends. The fusion of a single or two zymospores in the spore is the cause of the half-ring or full-ring appearance it takes on due to the dyes, the protoplasm of the zymospore diffusing to the periphery of the spore. This phenomenon is not specific to malaria mold spores. It is observed in most non-basophilic mold spores.

Refringent spores are formed at the end of very fine sinuous or spiral lateral branches of the mycelial filaments; on the other hand, free refracting spores germinate after having agglomerated several zymospores and emit a straight, sinuous or spiral filament, which will later form zymospores. In scanning the preparations, a large number of these spores are observed in germination or provided with a spiral pedicle; one of them is visible a little below the center of Figure 4; in the preparations of cultures of the pathogenic mold, the existence of refracting or basophilic spores is noted, emitting either an identical spiral filament or a curved and dilated filament having the appearance of the falciform bodies of the sporoblasts.

The germinal filament emitted by the spores constitutes the flagella whose formation has been observed on the gametes (germinal masses), forms of the hematozoan. These flagella are considered as spermatozoa of the gametes and these, consequently, as male elements.

Now this flagellum, or germinal filament, is born as much on basophilic spores as on non-basophilic spores and also on independent isolated spores in the blood; if flagella are formed on certain gametes, it is because these, which are agglomeration masses, or

germinal masses, contain spores which germinate by emitting a flagellum, even when included in a germinal mass. It is a general phenomenon and has nothing to do with sex; it is so general that we observe elements of the type of these gametes equipped with flagella in the normal spinal cord of the ox (Fig. 1, pl. 73, germinative mass emitting a multitude of flagella) and in the normal cerebellum of the sheep (Fig. 5, pl. 73); it is the phenomenon of the germination of spores gathered in an agglomerated mass in an in vitro mold culture (Fig. 2, pl. 73, *Mucor corymbifer*).

The formation of flagella emerging from the gametes is therefore a phenomenon of germination and not a phenomenon linked to a future sexual act. This assertion is also corroborated by the very nature of the gametes which are germinative masses in formation and not a protozoan in evolution. Let us add that it is above all the presence of these flagella which led to the parasite observed by Laveran being considered a protozoan.

4. Zymospores

These are fine spores, generally 0.2 to 0.4 µm, sometimes larger, basophilic, round, carried by very short pedicles, born either on the germinal filament of a spore, or on a multitude of fine, often spiral, filaments emitted by the mycelial branches. Once released, they are animated by Brownian motion and constitute the ferment of the malaria mold, or toxin of the pathogenic agent; they are the agent of the fermentations that take place in the blood of the malaria patient and of the fever that accompanies them; in preparations it is only possible to discern the origin of those that are born on the germinal filament of the refractile spores; for free zymospores, the distinction would only be possible by specific staining. Zymospores are the known granules that have been observed in parasitized red blood cells.

5. In blood preparations, *agglomeration masses or germinative masses* of all sizes and in very large numbers are observed. They are, moreover, reported and known; some, very large, formed of spores of various sizes and especially of refractile spores of 1 ½ to 2 µm, are those which are described as closely resembling amoebas.

The germinative masses must be classified:

A. The so-called division forms of the parasite. These are not division forms, they are agglomeration forms of refractile spores and zymospores *not yet fused.*

B. The rosette, or daisy, or raspberry, or morula bodies, which are not bodies in the process of dividing, but agglomerations of large, still distinct refractile spores, the peripheral teeth of which are often formed by the starting point of the spore's germinal filament. Several of these bodies, whole or fragmented, exist in figures 1, 3, and 5, pl. 293.

C. The gametes, germinal masses in the process of fusion.

D. The bodies considered to be the adult parasite, which are the germinal masses of the mold that have reached their complete development.

Further details are superfluous, since it is easy to isolate the pathogenic mold from the blood and these in vitro cultures contain:

A. The elementary form of Laveran's hematozoan, which is a refractile spore of the pathogenic mold developed either in the gonidia or in the mycelium.

B. The characteristic gonidia contained in the blood of malaria patients.

C. Agglomeration masses analogous to gametes and with amoeba-like shapes.

D. Formed germinative masses identical to the perfect hematozoa.

E. A mycelium bearing sessile or terminal lateral gonidia and sterigmata identical in shape to those contained in blood.

Isolation of the pathogenic mold.

The mold is easily isolated by the methods described many times: culture of blood in broth kept at 18-24°C, or on agar, carrot, etc.

It was a broth culture, inoculated with two or three drops of malarial blood, which gave me, in a few days, first a cloudy culture, then a greenish-yellow veil of the pathogenic mold; when subcultured onto solid media, agar, carrot, casein, etc., this veil gave rise to a short, greenish-yellow mold, so characteristic that it cannot be confused with any other; spread on a glass slide and examined under a microscope, its appearance and characteristics are also so distinct that it is easily recognizable. Its development is very slow on the most diverse media where I have cultivated it.

Microscopic examination of the mold demonstrates the presence of the following elements identical to those of blood:

1. Sterigmata and Gonidia.

The mold is of the monosterigmat gonidial type; at certain points, two or three sterigmata arise from a common, very short appendage (Fig. 1 and 2, pl. 294). The sterigmata give rise to strings of gonidia of a slightly variable oval shape, but most often one end is slightly acuminate, while the other is truncated.

The mold contains a large number of spherical perithecia not shown in the figures; there are also some of an irregular shape.

It is indeed in this gonidial form that the malaria agent vegetates in the blood, because we observe in the latter sterigmata identical to those of the in vitro culture, and bearing the same gonidia.

2. Intramycelial spores.

Many myceial filaments of the mold contain round spores, often in a continuous string (Figs. 4 and 5, pl. 294). These spores are refractile, not basophilic, and are generally 1 µ.5 in diameter; two of these spores, expelled from the mycelium, exist in Figure 5 to the right and left of the vertical spore-bearing filament. It is these refractile spores that are considered the first stage of evolution of the hematozoan; their nature is therefore precisely fixed here.

3. Germinal masses, gametes, rosette bodies, etc.

The refractile spores constitute, by their agglomeration, together with the zymospores and probably also with whole gonidia, elements which, after fusion, will be germinal masses; These elements present variable forms which have been considered as diverse forms of the evolution of the malaria haematozoan (male and female gametes, flagellated gametes, dividing bodies, rosette-shaped bodies, daisy-shaped bodies, raspberry-shaped bodies, morula-shaped bodies, amoeba-shaped bodies, etc.). Fig. 6 of Plate 294 contains two of these elements joined together (left of centre) which are agglomeration masses or germinative masses in the process of evolution; they are constituted by refractile spores, basophilic spores and basophilic zymospores; this aspect corresponds to that of the designated forms: amoeba-shaped bodies, raspberry-shaped bodies, morula-shaped bodies, etc.

This same figure also contains two other crescent-shaped agglomeration masses (in the upper half and on the right) (crescent gametes). By going through the preparations, we can find all forms of gametes, including gametes with flagella. We see therefore that these elements, male or female gametes, flagellated gametes, crescent bodies, have nothing sexual and that the sexuality which has been attributed to them is purely imaginative: the flagellum, which is the initial germinal appendage of the spores, is born on them as well when they are isolated; if we observe what it becomes subsequently, we see that it lengthens and forms zymospores; it is therefore a mycelial filament. A number of these spores emitting a spiral filament bearing zymospores are visible in Figure 5, Plate 62 (lower left corner), representing an irregularly shaped perithecia of the malaria mold. The sclerotia of the malaria mold are formed, like those of all molds, by agglomeration of basophilic and non-basophilic elements (germinative masses), and there is no sexual distinction to be made between these masses.

4. Oocysts, sporoblasts, falciform bodies or sporozoites.

It is accepted that the sexual development of the hematozoan takes place by male and female gametes of the parasite which, penetrating the digestive tract of mosquitoes, form oocysts, and these form sporoblasts which emit falciform bodies.

I have not studied the evolution of the malaria mold in the digestive tract of mosquitoes, this study being rendered useless by the determination of the nature of the pathogenic agent of malaria; being a mold, this agent is transmitted or inoculated by zymospores, spores and gonidia, or by any bacterial element contained in the cultures, this as for any other pathogenic mold; sexual reproduction in the body of the mosquito, which would be necessary for the transmission of the disease, has no basis from this

point of view; a mosquito that has bitten a malaria patient can certainly inoculate the disease into the man it bites five minutes later and certainly with more certainty than if it bit him forty-eight hours later without having bitten other patients.

Nevertheless, the evolution of the hematozoan described by Schaudinn and Luhe in the body of the mosquito, seems to correspond well to the evolution of elements characteristic of the malaria mold; the oocysts described by these authors must be the spherical perithecia that are found abundant in cultures of the mold; the sporoblasts are the large spores that form in these perithecia.

In in vitro cultures of the mold on carrot, for example, we find not only perithecia (oocysts), but also isolated sporoblasts; Figure 3, Plate 294, represents an element identical to one of these sporoblasts, because it emits bodies identical to the falcate bodies (in the center of the figure); these are, obviously, spores of a particular, lanceolate shape, a type of spore that exists in the most diverse forms in the perithecia or asci of the organic mold of many plants, grasses for example.

The observations of Schaudinn and Luhe therefore correspond to special forms of the evolution of the malaria mold, but in no way signify a sexual evolution of the hematozoan in the digestive tract of mosquitoes; they simply observed the evolution of the mold characterized by the formation of perithecia and the evolution of the spores that these contain; as for the explanations of these authors concerning the evolution of microgametes or macrogametes, the phenomenon of emission, by the macrogamete, of a pseudopod which would seize a microgamete in passing to bring about fertilization, they are very ingenious, but purely hypothetical and imaginary.

The fact that the pathogenic agent of malaria is a mold makes evident the inaccuracy of the complicated description or theory of the evolution of the hematozoan.

The evolution of the mold is complete in the body of the malarial person and has no need of the mosquito as an intermediate host; the latter is only the agent of transport and inoculation of the virus.

5. Formed germinal masses.

The formed germinal mass is the one that has acquired enough basophilic elements to enter into germination; at the beginning, when it contains little more than refractile spores, it is poor in basophilic protoplasm and corresponds to a male gamete; at its complete development, when it has agglomerated and fused enough basophilic elements, it constitutes the female gamete.

The study of the evolution of the germinal mass, made in this work, has shown that its enrichment in basophilic protoplasm (or chromatine) takes place by the contribution and fusion of sporiferous or basophilic bacterial elements, initially foreign to the mass. The distinction of gametes into male or female by the quantity of chromatic protoplasm they contain is therefore an error; the male gamete, less rich in chromatic protoplasm than the female gamete, is a stage, less advanced than the latter, of the germinative mass.

In in vitro cultures of the malaria mold, we find, *as in all mold cultures,* different stages of the germinative mass that could be taken for either male or female gametes. These masses do not exist in large numbers in a perfectly distinct state, because they disintegrate easily. They are found, however; Figure 2, Plate 294, shows one of them (top) that could be considered a female gamete; it is rigorously similar, as can be seen, to the element that is considered the perfect hematozoan.

* * *

From this parallel study of the elements of the malaria mold in the blood and in in vitro cultures, we can conclude firstly:

1. The various abnormal elements that exist in the blood of a patient suffering from malaria, at the time of a febrile attack, *are all found, in an identical form, in the in vitro culture of the malaria agent.*

2. The culture obtained is therefore that of the malaria agent.

3. This culture contains, in vitro, the characteristic elements considered to be the forms of Laveran's hematozoan, notably the elementary form (spores), and the forms called gametes, crescent bodies, raspberry bodies, morula, etc.

4. The pathogenic agent of malaria is therefore a mold and not a hematozoan.

5. The most characteristic elements of pathogenic mold in the blood of patients at the time of the crisis are not the forms designated under the name of hematozoa; they are the oval gonidia, the sterigmata often bearing an oval gonidia, and mycelial filaments bearing these same sessile gonidia.

The study just presented demonstrates, on the other hand, that:

1. The complicated cycle described for the evolution of the malaria agent does not exist.

2. The mold that is the pathogen of malaria evolves, like all molds, through a cycle of four successive transformations:

1. agglomeration of bacterial elements (spores, zymospores, bacilli, etc.);
2. fusion of the agglomerated elements to form germinative masses;
3. germination or mycelial vegetation of these masses;
4. formation of spores, gonidia, and segmentation of the mycelial filaments into bacterial elements

3. The evolution of the mold does not require its passage through an intermediate host, the mosquito; this insect is only the inoculation agent; this being carried out solely by spores, zymospores, or other bacterial elements, the mosquito can inoculate the disease into a healthy person immediately after biting a sick person, if the latter's blood contains the appropriate elements. We will see later that the usual mode of contamination or inoculation of the disease is certainly not the latter.

Identification of the pathogenic mold.

In order to identify the malaria mold, I researched and obtained, either by direct culture of the blood of a malaria patient, or by culture of the organs of a rabbit inoculated with this blood, or by transformation of isolated molds:

1. A cream-colored or yellowish mold presenting, on certain mycelial filaments, the gonidial form *Monilia* (Fig. 1, pl. 295); on others, a form *Acremonium alternatum* whose spores, instead of being oval, are in the form of cylindrical and linear rods (Fig. 2, pl. 295); the sterigmata of this last gonidial form have retained a form similar to those of the form *Monosterigmata* (Fig. 2, pl. 294); these are therefore forms derived from the latter and, probably, from its evolution towards the form *Penicilium.*

2. A milk-white mold, poor in fruiting bodies, still possessing very rare sterigmata bearing an oval gonidia, and whose mycelial filaments give rise to numerous thin spiral filaments ending in a fine spore.

3. The *Aspergillus* form (Fig. 4, pl. 295).
4. The *Penicilium* form (Fig. 5, 6, 7, 8, pl. 295).

These four forms were obtained by inoculating the rabbit with malarial blood taken during an attack and subsequent culture of the pulp of its organs on agar.

A bacterial culture of malaria mold develops on cultures of the pulp of the organs of inoculated animals, in the form of a whitish coating that runs to the bottom of the tubes. This culture is made up of two different elements: 1st short, wide, basophilic bacilli (approximately 0.6 out of 24), segmenting into two shorter oval bacilli and then

into round granules; 2nd very short, wide coccobacilli, barely stainable by the dahlia. I recognized a complete identity between the bacterial culture of rice (obtained by inoculating an emulsion of crushed rice grain in broth) and that of malaria mold. It is made up, like the latter, of short basophilic bacilli and short, wide coccobacilli, barely stainable by the dahlia.

Following this observation, the *Penicilium* form of the malaria mold was compared to that of rice. I observed between these two forms an identity similar to that of cultures. This double identity is sufficiently clear to be able to conclude at present that, in the case studied here, the malaria mold is a gonidial form of the organic mold of rice.

This conclusion is supported by the nature of the lesions caused in the guinea pig by the inoculation of bacterial cultures of the two molds. In both cases, the liver of the animals is cirrhotic, pale, and very friable. The bacterial culture that I obtained from rice is less virulent than the culture of the malaria mold; the former kills in about fifteen days, the latter in six to eight days; but there are gonidial forms of the latter that kill only in three or four weeks. Virulence therefore depends, here, on the gonidial form.

Currently, new gonidial forms of rice mold and malaria mold are being sought, mainly to verify whether the characteristic *Monosterigmata* form of the latter is found among the gonidial forms of rice.

I should point out that this identification applies only to the case studied and to similar cases. Three different malaria agents have, in fact, been described; some have argued that there is only one malaria agent; others believe that there are several. It is likely that there are several and that these are the bacterial cultures of the organic mold of plants belonging to species of the same family or of neighboring families.

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The example provided by the Gramineae is very illustrative; the food plants of this family are the pathogens of paratyphus A (wheat), paratyphus B (rye), typhoid fever (corn), and exanthematous typhus (oats). Among the commonly cultivated grasses, only barley causes a distinct disease (diphtheria). It is therefore not surprising that rice causes malaria, a disease related to the different species of typhus. But bacterial cultures of other plants must cause malaria, because this disease is contracted in marshy regions where rice is not cultivated.

Malaria is known to be contracted only in marshy regions; being caused by the bacterial culture of organic mold on plants, it is therefore plants growing in marshes that are its agents. Now, marshes are always covered with plants that grow in damp places, on mud, and which belong to the grasses and to a very related family: the Cyperaceae; these are mainly the two genera Carex and Cyperus, whose species populate the mar-

shes with countless individuals; at a given moment, these plants rot on the spot and we can say that the mud of the marshes is made up of their rot; this mud is therefore rich in bacterial culture of plants of the Cyperaceae family and in particular of species of the genera Carex and Cyperus.

The mosquitoes that prefer to inhabit marshes land on the mud, probably feeding on the putrefying organic matter it contains; in any case, they are contaminated by this matter and can inoculate humans with it.

I directed my research toward the study of spontaneous grasses and Cyperaceae in order to isolate bacterial cultures and gonidial forms of organic mold from the most widespread species.

* * *

In concluding this study of malaria, it is useful to recall the significance of the particular mode of evolution of this disease relative to the mechanism of the phenomenon of vaccine protection. The mold that is the pathogenic agent of the disease evolves in individuals in the permanent mycelial form, the elements of which are found during attacks, a fact demonstrated by the presence, in the blood of patients, of mycelium, sterigmata, and gonidia characteristic of the mold.

The febrile attack is due to a vegetative growth of the pathogenic mold accompanied by the formation of a large quantity of zymospores.

The repetition of attacks demonstrates that the establishment of the permanent mycelial vegetation of a mold, which creates the vaccinal state for certain pathogenic agents, does not create it for all. The vaccinal state exists only relative to the acute or fermentative phase of a disease; it cannot exist against the mycelial phase, since it is the latter that creates it.

Since malaria is characterized by a repetition of the acute or fermentative phase at distant intervals, it must be concluded *that vaccine protection against the acute phase of a disease is not a general fact,* but only a fact specific to certain species of pathogenic agents, linked to the special characteristics of their development. It has also been previously stated that vaccine protection does not depend at all on a reaction of the organism, but only on the development, in the organism and by the pathogenic mold, of the elements which ensure its mycelial vegetation.

SLEEPING SICKNESS. TRYPANOSOMA GAMBIENSE

The study of the pathogenic agent of this disease was carried out on guinea pigs made available to me by Professor Auguste Pettit of the Pasteur Institute, which he had inoculated with Trypanosoma gambiense, from a missionary from equatorial Africa suffering from sleeping sickness.

The study carried out on these animals, and on others successively inoculated with the blood of the former, led to the knowledge and isolation of the pathogenic agent of the disease; it is a mold from which I obtained four gonidial forms. Trypanosoma gambiense is a mycelial formation of this mold; it is therefore not a protozoan.

Study of the elements of the pathogenic mold in the blood of the inoculated animals.

On one of the first inoculated guinea pigs, I was first able to observe that the blood contains:

1. Large germinative masses in vegetation, emitting mycelial filaments ending in dilations which are trypanosomas.

2. Spores of 0.8 to 1 μm bearing a flagellum which is a germinative filament.

Germinative masses can be observed in the blood obtained by pricking the ear. A group of two or three of these masses is represented in figures 1, 2, 3, of plate 9. The preparation used for these photographs was strongly colored by dahlia; but this coloring is not the most favorable for observing trypanosomas; nevertheless, their presence can be seen in the figures In Figures 1 and 2, there are two large, joined germinative masses, each emitting a bundle of mycelial filaments B, C; the filaments of bundle B, emanating from the germinative mass A, separate at D; by examining the end of the filaments with a magnifying glass in Figure 3, we will see, at F, dilations which are trypanosomes. Certain mycelial filaments, shorter than these, form dilations or trypanosomes in the vicinity of the germinative mass, at E, for example (Fig. 1).

It is these dilations of the mycelial ends which, when detached, constitute the trypanosomes isolated in the blood; the flagellum that these bear is the mycelial filament that attached them to the germinative mass.

Another demonstration of this fact will be given later.

When we examine blood diluted with salt water, between the slide and the slide, we note the presence, between the blood corpuscles, of very mobile elements equipped with a flagellum animated by lively movements; because of this movement and the displacement of the blood corpuscles, we cannot clearly distinguish the shape of these elements; but if we examine the corpuscles in a region where they are distant from each other, we note that some of them carry one, two, or more of these flagella animated by movements and appearing to emerge from their interior.

In preparations made by rapid desiccation of diluted blood and stained strongly with dahlia, we see that these elements are round spores of about 1 u, bearing a flagellum, and which come to stick to the surface of the globule or penetrate inside, the flagellum remaining free on the outside. In the interval between the globules, we observe these isolated spores equipped with their flagellum. One of these spores parasitizing a blood globule is represented in Figure 2, Plate 296; five or six other spores parasitize the same globule; the flagellum of two of them is slightly visible outside the globule; the flagellum of the others is applied to the body of the latter. This figure relates, not to one of the animals inoculated with the original human virus, but to an animal in which the disease was reproduced by inoculation with the pathogenic mold; The flagellated spores thus photographed are, moreover, rigorously identical to those observed in the blood of animals inoculated with the virus from the blood of contaminated humans.

Isolation of the pathogenic mold.

This isolation is obtained by culturing on agar the blood and pulp of the organs (lungs, liver, spleen, lymph nodes) of animals killed about three weeks after inoculation, or of animals that died of the disease. In these cultures, I obtained four gonidian forms of the pathogenic mold.

1. *Sporothricum* (or *Ovularia)* form. This is a salmon-pink mold, low in height, with rapid growth (Fig. 4, pl. 296).

2. *Aspergillus* form (Fig. 3, pl. 296).

3. *Sterigmatocystis* form (Fig. 5, 6, pl. 296).

4. *Penicilium* form. I obtained this form by culturing the pulp of guinea pig organs inoculated with the human blood virus (Figs. 1, 2, 5, 6, 7, pl. 296). I obtained it, on the other hand, in cultures of the pulp of guinea pig organs inoculated with spores of the *Sporothricum* form (Fig. 4). This fact demonstrates that these two gonidial forms are related to each other and belong to the same original species.

Experimental reproduction of the disease by inoculation of the pathogenic mold.

The four gonidial forms of the pathogenic mold inoculated into a guinea pig kill it in ten to twenty-five or thirty days. The *Aspergillus* and *Penicilium* forms appear to be the most virulent. The *Sporothricum* form kills the guinea pig in fifteen to twenty-five days, causing essentially the same lesions in the animals as the virus originating in humans.

This gonidial form probably corresponds well to the one that vegetates in the organisms of sick humans and animals, *because the characteristic elements of sleeping sickness are found in the blood of the animals into which it is inoculated: the trypanosome, the flagellated spore, and blood cells (most of them) parasitized by this spore.* Figures 1 and 2 of Plate 296 represent:

Figure 1: A germinative mass of the pathogenic mold emitting mycelial filaments that expand, taking the form of the trypanosome; one of them has such a characteristic shape that the slightest doubt can be preserved; Figure 2: A blood cell parasitized by flagellated spores.

A large number of flagellated spores are found in the blood, but, on the other hand, trypanosomes are rare.

I have not been able, so far, to identify the pathogenic mold. It is possible that this identification can only be made in the region where the disease develops in equatorial Africa, and this for the following reasons:

The original source of the pathogenic mold is necessarily an animal or a plant: it is therefore the organic mold of this animal or this plant that must be possessed to be able to identify the pathogenic mold, this organism certainly only living in regions where sleeping sickness develops.

We know that the disease is inoculated by the bite of the tsetse fly. But it is likely that inoculation most often takes place not with the blood of patients bitten by the fly, but with the original virus taken by the fly from a living animal or from a living or decomposing plant species.

The first patient to be infected in a region must have contracted the disease in some way other than by inoculation of the blood of another patient by the tsetse fly.

It is likely that the tsetse fly contaminates humans not primarily because it transmits the disease from one human to another, but because it *habitually* bites an animal widespread in equatorial Africa and whose blood constitutes its daily food, or because it habitually lands on rotting plant matter on which it feeds.

* * *

The facts just established for sleeping sickness apply to all other trypanosomiases that affect humans or animals: *Trypanosoma rhodesiense* or trypanosomiasis, *Schizotrypanosis* (Chagas disease), Surra, Nagana, Caderas disease, etc. These diseases result from the inoculation of humans or animals by a biting insect with the organic mold of an animal or plant, whose blood or sweat frequently constitutes food for it.

The nature of trypanosomes, vegetative elements of a mold and not protozoan organisms, indicates:

1. That the disease is transmitted as well by the other elements of the mold, spores, bacterial elements, germinative masses, as by the mycelial element called trypanosome.

2. That the cycle of evolution and the mode of reproduction of trypanosomes that have been indicated are imaginative. A trypanosome is born solely from a germinative mass; this is not a stage in the evolution of the trypanosome, but the essential element in the development of the pathogenic mold which, later, will give rise to trypanosomes by germinating; these *Leischmania* or *schizogony* forms of *Schizotrypanum Cruzi* are not stages in the transformation of the latter, but germinative masses of the pathogenic mold. The evolution in the body of the biting insect (*Triatoma megista*) cannot be different from the evolution in the human organism; there are other elements of the pathogenic mold in the insect and in humans that have not been seen by observers (mycelium, fruiting bodies, bacterial elements, spores).

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THRUSH

I studied three cultures from one case of thrush in an infant and two cases of thrush in an adult.

The creamy matter of the thrush was cultured on agar and on carrot.

From one of the cultures, I obtained, by direct transformation, a mold of the *Rhizopus nigricans* form. From the culture of another case, I obtained the *Penicilium* form by direct transformation. Finally, I obtained the *Penicilium* form from all three cases by the indirect route, by inoculating the thrush culture into the guinea pig and culturing the pulp of the organs after death. The *Penicilium* form thus obtained by the indirect route is shown in Figures 1 to 16 of Plate 298.

The *Penicilium* and *Rhizopus nigricans* forms are sufficient for the identification of thrush. The *Penicilium* form is that of the organic mold of beef; I obtained the *Rhizopus nigricans* form by culturing cow casein; it even develops on casein heated to 100°C for 20 minutes; it developed on three samples of casein (out of six prepared with the same

material) which had been heated for 20 minutes at 115-120°C, but after only four to five months.

On the pulp of normal beef tissues (lung, liver, spleen, brain, muscles) another form, *Rhizopus* (Fig. 14, 15, 16, pl. 178), develops. It is black-brown in color, the gonidiophores of which are solitary and of the same length (3 to 5 millimeters) as those of *Rhizopus nigricans*; it appears to be a form of the latter.

Thrush is therefore a lower vegetative form of the organic mold of beef. The subjects from which it was taken were fed partly with cow's milk. It is therefore this milk that develops thrush in the mouth through the elements of the organic mold of the cow that it contains; the small quantity of milk that remains enclosed in the mouth between the cheeks and gums is sufficient to ensure the development of the mold.

I have not studied other cases of thrush, but it is likely that other forms exist, and that human milk and goat's milk are likely to determine the development of two of them.

Washing the infant's mouth immediately after ingesting the milk can therefore prevent the development of thrush.

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FURUNCULOSIS. STAPHYLOCOCCUS.

The term staphylococcus refers to a bacterial element appearing in the form of isolated, rounded grains. From what has been explained so far, it is clear that the word staphylococcus cannot be an exact generic designation, because it can apply to a considerable number of different species, for example, to a grain-shaped culture originating from the organic mold of a plant species as well as to a grain-shaped culture originating from the organic mold of an animal species.

We will only discuss human staphylococci, which are known to be encountered in a large number of conditions.

Staphylococcus pyogenes aureus, isolated in its pure state from a boil or carbuncle, can be easily transformed into various gonidial forms of the mold that gives rise to it by inoculating it into a guinea pig and culturing the pulp of its organs. Transformation can also be achieved directly by transferring a culture of staphylococcus on agar to various solid media (carrot, casein, beef, sterile dung, etc.). By these two methods, I obtained the transformation into *Penicilium* (Fig. 3, 4, 5, pl. 307) and *Aspergillus* (Fig. 1, 2, pl. 307) forms.

I obtained, also by direct transformation, the *Aspergillus* form of *Staphylococcus* albus (Fig. 7) and *citreus* (Fig. 6, pl. 307).

The *Penicillium* form of *Staphylococcus pyogenes aureus* is identical to that of human organic mold; *Staphylococcus aureus* is therefore a bacterial form of hu- man organic mold, a fact which leads to the following conclusion: *furunculosis is an autogenous disease of humans, and not a disease caused by a heterogeneous germ.*

This conclusion does not imply that the disease cannot develop by cutaneous autoinfection.

As in plants, animal organic mold eliminates bacterial elements (spores, zymo- spores), notably through the sebaceous or sweat glands, through the hair follicles. These elements can vegetate in the cavities of these glands or follicles and multiply there. They spread on the surface of the skin and can, when they have acquired sufficient vegetative activity, can cause the formation of boils in affected follicles or glands; they can also infect neighboring glands or follicles that are still unaffected.

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Knowledge of the nature and original source of *Staphylococcus pyogenes aureus* enables us to appreciate the value of vaccination and serotherapy trials against furunculosis. *Staphylococcus* being an element of the organic mold specific to the human organism, it becomes obvious that wanting to vaccinate against it is impossible, since man cannot be vaccinated against his own organic mold.

But, even if the serum of an immunized animal species, the horse, for example, had a curative action, which seems impossible, its use should be rejected without hesitation, because its injection would confer on man the mycosis of the organic mold of the horse, the remote effects of which are still little known, this to avoid or only shorten a temporary and minor illness, furunculosis.

It is therefore necessary to conclude:

1. That vaccination against furunculosis is impossible.

2. That serotherapy should not be used against furunculosis because, even if it were effective, it would expose the patient to accidents much more serious than those of the disease being treated.

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STREPTOCOCCUS

Like the word staphylococcus, the word streptococcus cannot have any generic or specific meaning. A streptococcus is a chain of rounded grains or cocci. It is not, as is commonly believed, a series of cocci grouped into chains, which implies an attraction or adhesion between the elements that would determine their grouping; a chain of streptococci comes from a single formative mycelial filament segmented into rounded

elements; this formation of chains is easy to observe by following cultures; at a certain point, we can see that the formative filament is undivided, then often segments first into bacillary elements which, themselves, while remaining united, segment into grains. Often, we see in the same chain that one part is formed of bacilli and another of cocci Formerly, we see a long mycelial filament comprising a long portion still undivided and the rest segmented into a chain of cocci. In such a filament, the cause of the chain arrangement is therefore very apparent.

The formation of streptococci is identical to that of chains of bacilli or that of chains of coccobacilli, of which various examples will be found in this work.

The organic mold of many animal or plant species can give rise to the formation of chains of cocci or streptococci; the organic mold of humans has a form, such as that of carrots, for example.

There is therefore neither a genus nor a species of *Streptococcus*, but only a streptococcus arrangement or bacterial form; this form cannot be specific, because the molds that present it simultaneously possess other bacterial forms.

The streptococcal system is, moreover, only a transitory state, the segmentation of the mycelial filament into cocci being followed by their separation into isolated elements, then their subsequent reunion into clusters which will constitute germinative masses.

A streptococcus is therefore a transitory state of the segmentation of the mycelial filaments of a mold.

These considerations explain why I did not apply myself to transforming cultures of streptococci into gonidian forms of the mold which gave rise to them.

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I only carried out this operation for a single culture of streptococcus in broth from the laboratory of Doctor Bezançon, professor at the Faculty of Medicine. This culture, preserved at room temperature (18-20°), spontaneously gave rise to a veil which, transferred to agar and carrot, transformed into a milky white mold of the gonidian form *Monosterigmata* (Fig. 9, pl. 308); this form was evolving, because we already observed groups of sterigmata in an umbel.

I have not pursued the identification of this mold, but I point out that this gonidial form appears identical to one of the gonidial forms of carrot, and that bacterial cultures of the latter contain chains of cocci; bacterial cultures of carrot cause in rabbits, by cutaneous inoculation by means of multiple light superficial scarifications, a bright redness with swelling of the skin quite similar to a patch of erysipelas. This fact encourages us to investigate whether certain forms of erysipelas could be due to accidental inoculation of a bacterial culture of carrot.

The origin of the chain of the streptococcal apparatus, the fact that this apparatus is found in a large number of molds, explains why we have been led to admit that there are multiple races of streptococci; molds that only undergo rudimentary mycelial development, generally due to the poverty or unsuitability of the medium in which they live, are those which, when brought back to the bacterial form in a broth culture, or on their own culture medium, most easily give rise to the formation of chains of cocci, a process which constitutes their mode of multiplication; most organic molds of animals and plants can be brought to a form of this kind.

The streptococcal form can be obtained by preserving animal serum for a few days at laboratory temperature. When flakes form in it, these, examined under high magnification, often show a streptococcal form of the organic mold.

By this process I obtained a streptococcal form of the horse's organic mold that bears a striking resemblance to Schütz's streptococcus; if this identification were confirmed, it would establish that horse strangles is a disease of its specific organic mold.

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There is therefore not one genus Streptococcus, but multiple species types of molds that can present the streptococcal apparatus in their bacterial culture. In these cultures, the mother organic mold generally gives rise, alongside the streptococcal apparatus, to other multiple bacterial forms, bacilli and coccobacilli. This is an important fact to remember.

On the other hand, a pure culture of streptococcal form can easily be transformed into other bacterial forms:

1. By inoculation in sweetened peptone veal broth maintained at 20-24°.

2. By inoculation in the guinea pig and culture of the organ pulp on agar; this pulp being inoculated on the upper part of the agar only, we see developing below, on the uninoculated region, a semi-liquid culture which often flows to the bottom of the tube and which is a bacterial culture of the mother mold of the streptococcus.
This culture or its subculture in broth may contain the streptococcal form alongside other bacillary forms.

From these facts, it must be remembered:

1. That the fact of encountering a streptococcal form alongside other bacillary forms does not prove that there is an association of different bacterial species; they may belong to the same pathogenic mold.

2. That in the case where this multiplicity of forms and not of different species is observed, the virulence of the culture is, most often, its particular property and does not result from a non-existent association of species.

3. That there are a considerable number of cultures containing the streptococcal device and which are specifically different; there are streptococci of the organic mold of most animals and plants.

4. *That these cultures, which are specifically different, cannot exert a vaccinating action against each other.* The vaccinating or curative action of a serum could only exist against the culture that has developed the vaccinating properties and can only be ineffective against other cultures that are specifically different.

These facts explain why all attempts made so far to obtain an antistreptococcal serum have failed. It is easy to see that attempts to obtain a polyvalent serum are doomed to such certain failure because of the multiplicity of pathogenic species that can present the streptococcal apparatus. The application of such sera to humans is justifiable only in the case where the identification of the pathogenic streptococcal form has been made, an operation which will at the same time determine whether the serum, prepared with a specifically known culture, is specifically applicable to the disease being treated.

PYOCYANIUM BACILLUS

(Pseudomonas aeruginosa)

I obtained the direct transformation of a culture of a pyocyanium bacillus by transferring it to egg yolk; it developed the *Penicilium* form of the parent mold (Fig. 1, 2, 3, pl. 308). I have not yet made a sufficient identification study of this *Penicilium;* I only indicate here that it presents the characteristics in common with the *Penicilium* form of the organic mold of corn, which multiple identification research indicates as being similar to that of the typhus mold. Now it is known that the presence of the pyocyanium bacillus has been observed several times in the organs of patients suffering from typhoid fever.

On the other hand, the bacterial culture of corn can appear in the form of a pyocyanic bacillus, that is, a greenish-colored culture, or in the form of cultures without a distinct coloration. The bacillary form contained in these cultures appears identical to that of the typhus bacillus and at the same time bears a strong resemblance to the pyocyanic bacillus. As this subject is currently under study, I will limit myself here to reporting the facts observed.

I must add that the facts thus observed result from a coincidence, because the types of pyocyanic bacillus are multiple; they can be obtained by reducing the organic mold of plants to the bacterial state, particularly that which is taken from the organs provided with chlorophyll: leaf, petiole, green bark of the stem. I obtained one from barley, another from leek, a third from parsley, etc.

BACILLUS SUBTILIS AND MICROCOCCUS CATARRHALIS

These two germs appear here only because they were used to demonstrate the absolute generality of the nature of bacterial cultures (Bacillus subtilis (Fig. 4, pl. 308); *Micrococcus catarrhalis* (Fig. 7, 8, pl. 308)). I have made no attempt to identify them, given their secondary interest. *Micrococcus catarrhalis* appears to be a bacterial form of the organic mold of man.

The *Penicilium* forms of these two bacterial cultures were obtained by direct transformation by transferring them to various solid media.

I have also transformed other bacterial species from which I have obtained the *Penicilium* form, tetragene citrin and tetragene zoogleic for example.

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BREWER'S YEAST

The transformation of brewer's yeast into molds of various gonidial forms is discussed here because it relates to a most important question: that of the nature and origin of yeasts.

Various experimenters have maintained that yeasts are related to more developed fungi; according to Jörgensen and Sorel, the gonidia of *Aspergillus oryze*, which is used to make sake, a Japanese drink, are related to true yeasts. This conclusion was contested by other observers. According to Viala and Pacottet, *Glæosporium ampelophagum* and *Glæosporium nervisequum* could transform into true yeasts, producing alcoholic fermentation when cultivated in very sweet environments. The results of these authors' experiments were contested and attributed by Guilliermond to impurities contaminating the fungi tested.

The conclusions of Jörgensen, Viala, and Pacottet are, however, justified. Yeasts are one of the first forms taken by the organic mold of a plant when it develops outside the organism or within the organism when it dies; they spontaneously transform into hyphomycetes of various gonidian forms, and these can reciprocally be returned to the yeast form by suitable culture.

The proof of these facts can be given with such certainty and ease that no dispute remains possible. First, by culturing a yeast on selected solid media, it can be transformed into molds of various gonidian forms. By suitable culture, certain higher molds of the *Penicilium, Aspergillus,* and *Mucor* forms can be reduced to yeast forms; for some, it is sufficient to cultivate them on a solid, but very humid, medium, on carrots for example, and at a suitable temperature (higher than the mold's optimum temperature).

But these processes are long. There is a faster one, with certain success, which allows us to establish the origin of the yeast and to determine the plant or animal organism that is its original source. This process involves inoculating the guinea pig with pure yeast and culturing the pulp of its organs on agar, either upon death or after a few days if it is saturated. Various gonidial forms of the mother yeast mold develop on the pulp of the organs, including, without exception, the *Penicilium* form.

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If, for example, brewer's yeast is used for this demonstration, a *Penicilium* form develops in the cultures in large green colonies from which it is easy to collect *pure spores and only spores*, with a wet stick lightly touching the surface of the culture. These spores being supported on the surface of a tube of highly glucose agar, the surface of the medium is completely covered with the *Penicilium* culture; this has retained the fermentative aptitude of yeast; it forms such a notable quantity of alcohol that its odor is strongly perceived by the vapors which pass through the cotton stopper, if only the cap which has been carefully placed at the orifice of the tube is removed.

Another demonstration can be given for grape yeast. I collected, from the surface of ripe grapes, yeast cells that normally exist on all grapes and inoculated them onto agar; after about two weeks, I obtained the development of green colonies of *Penicilium.* I also cultivated on agar the pulp of the subcortical tissue of green vine shoots, carefully decorticated so as not to soil them. One can also slab them on the surface and then take medullary tissue, the pulp of which is cultivated, or inoculated into the guinea pig after having crushed it in water; the organs of the animal are cultivated after death. One thus obtains the *Penicilium* form of the organic mold of the vine. This form is identical to that provided by the culture of yeast taken from the surface of the grapes.

The *Penicilium* form of the vine can also be obtained by burning half the surface of an intact grain, and removing from the grain, through the burned part, a portion of the pulp from the inside of the grain. This pulp, placed in a closed and capped aseptic bottle, without the addition of any culture medium, spontaneously molds there after a certain time and gives rise to the same *Penicilium* form as yeast.

I recall, moreover, that it has been demonstrated elsewhere:

1. That *Botrytis cinerea* (*Sclerotinia Fuckeliana*), to which gray rot of grapes is attributed, is not a heterogeneous parasite, but only a gonidial form of organic mold of the vine.

2. That *Botrytis cinerea* and *Plasmopara viticola* are the same gonidial form of organic mold of the vine, designated by two different names.

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3. That the *Penicilium* form of the organic mold of the vine develops directly on the mycelium of the *Botrytis cinerea* form.

Grape yeast is therefore a vegetative form of the organic mold of the vine; it develops, as Pasteur observed, both on the wood of the vine and on the grape bunches.

Thus is established not only the nature of grape yeast, but also its original source.

In all natural fermentations, the yeast or ferment is a vegetative form of the organic mold of the animal or plant whose organic matter is subjected to fermentation. This fact has been demonstrated in the study of ferments. It has been shown, in this regard, that *the active elements of fermentation are not ferment or yeast cells, but very fine spores (zymospores) animated by the Brownian movement,* passing through the filters, and formed by the germinal filaments of larger spores or by the mycelium of a mold derived from the organic mold of the plant or animal The ferments that form in living beings are all made up of the same active element: the zymospore, formed on the mycelial filaments or by other elements of the organic mold itself. It is therefore not the yeast cells, that is to say, its mycelial elements, that possess the fermentative power, but its zymospores.

* * *

Let us now move on to the specific study of brewer's yeast. I carried it out using pure yeast that Mr. Jenné, administrator of the Sochaux brewery (Doubs), kindly made available to me. I fairly easily transformed this yeast into hyphomycetes by the following processes:

The yeast was first inoculated in peptone, glycated veal broth, stored at room temperature; the culture produced a film after about twelve days. This film was inoculated onto various solid media: carrot, agar, meat, sterile dung, etc. After a few days, a gray, long-haired mold developed on carrot, which was a *Sporothricum* form, producing large fruiting bodies similar to yeast cells either along the mycelial filaments or at their ends.

On sterile dung, the film directly produced the *Penicilium* form.

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The original yeast was also inoculated into guinea pigs whose organ pulp was cultured on agar. The cultures gave rise to the *Penicilium* form and the *Aspergillus* form.

The *Penicilium* form, identical to that obtained on sterile droppings by the direct route, is shown in Figures 1 to 6 of Plate 306. Having been subcultured a first time on highly glucose agar, it is easily seen by the odor that it has vigorously retained the fermentative property of the yeast. To carry out the inoculation, with certainty of not collecting any elements other than the *Penicillium* spores, the original culture tube is placed vertically above a new tube and spores are dropped into the latter by striking the upper tube a few light taps.

The *Aspergillus* form (Fig. 7, pl. 306) does not ferment glucose. This fact explains the disruption caused to beer fermentation by the appearance in the vats of the yellow sarcine well known to brewers. This is a vegetative transformation of the yeast evolving, I believe, towards the *Aspergillus* type. Indeed, the *Aspergillus* culture turns yellow and gives rise, when it liquefies on the surface of the agar, to a yellow coating.

I have not done any special research to identify brewer's yeast; but two elements indicate that the one I have studied is a vegetative form of the organic barley mold. Indeed, the *Penicilium* form of this (Pl. 218) is identical to that obtained from brewer's yeast (Pl. 306). The *Mucor* form of the organic barley mold (Figs. 8 and 9, pl. 161) liquefies quite easily and gives rise to fruiting bodies of the same shape as yeast cells, or to mycelial filaments vegetating in the yeast form. A special study of this *Mucor* form will be able to clarify whether, as I believe, brewer's yeast is one of these lower vegetative forms.

I point out here that, by transforming the organic mold of barley, a *Penicilium* form is obtained that does not transform sugar into alcohol. This fact does not mean that any *Penicilium* that possesses this power cannot belong to the organic mold of barley. It has already been indicated elsewhere that the same gonidial form can be obtained by transforming two other very distinct forms, and that these molds, obtained from each of these two, can possess different fermentative properties although being identical in terms of the gonidial form.

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SCALP DISEASES AND DERMATOPHYTES

The dermatophytes, *Trichophyton, Microsporum, Achorion*, etc., are considered fungi forming a special class whose place in the classification has not yet been clearly established.
The genera *Trichophyton* and *Microsporum* do not possess distinctive characteristics that allow for a rational generic grouping.

The shape of their mycelium, their spores, their sporiferous hyphae, the existence of spiral filaments, formations that Sabouraut calls pectinate organs, have nothing special

about *Trichophyton* and *Microsporum*; these are characteristics that belong to all molds in general, at a certain stage of their development. Only the formations called spindle organs by Sabouraut, which do not differ significantly from the fructifications of *Achorion gypseum* and *Epidermophyton inguinale*, constitute a characterized gonidial form. These fructifications are, as I have previously demonstrated, a stage in the development of the fructifications characteristic of the gonidial form *Penicilium*; a large number of molds can exhibit the gonidial form of *Achorion gypseum*.

The *Trichophyton* and *Microsporum* forms are molds of the incompletely developed forms of *Monosterigmatis, Penicilium*, or *Aspergillus.*

When seeking to isolate dermatophytes by cultures of hair or epidermal scales, scabs, etc., very characteristic gonidial forms are most often collected at the same time, notably the *Aspergillus* and *Penicilium* forms, which are the perfect state of *Trichophyton, Microsporum,* and *Achorion.* Systematically, dermatologists have so far only collected molds of white, pink, ochre, and sometimes purple color, which constitute the group of fungi called dermatophytes, and have excluded from their studies all green molds, *which they considered to be banal and devoid of interest;* We will see later what considerable importance, on the contrary, the study of these latter presents.

I studied dermatophytes on pure cultures that Doctor Sabouraut kindly made available to me.

By examining these cultures carefully, one can sometimes recognize a rudimentary gonidian form; but it is easy to make them progress by sowing and transplanting them several times onto other media, very sweet agar (5% glucose, 5% maltose), meat, casein, sweet carrot, egg yolk, etc.

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By transplanting *Trichophyton gypseum asteroïdes* several times onto sweet agar or sweet carrot, for example, it is easily progressed to a definite gonidian form, the *Monosterigmata* form (Fig. 1, pl. 299), which could also be taken for a poorly developed *Acremonium alternatum* form. By this process, I obtained the direct transformation of *Achorion Schoenleinii* into *Penicilium*.

I easily obtained the *Penicilium* forms and, sometimes, other gonidial forms, of all the species of *Trichophyton, Microsporum, Achorion, Epidermophyton,* etc., given to me by Doctor Sabouraut. To obtain them reliably, it is sufficient to inoculate a guinea pig, to cultivate the pulp of its organs on agar after death, and to collect the molds that appear in the cultures from the third to the sixth or seventh day. Later molds must undergo more serious control with respect to the gonidial forms of the guinea pig's organic mold.

Since it is not possible to multiply the number of plates in this work too much, only a few of the cultures studied will be discussed here.

Trichophyton gypseum asteroides

Figure 1 of Plate 299 represents a slightly evolved form, a stage in the progression of *Trichophyton*; the *Penicilium* form was obtained by inoculating the guinea pig with *Trichophyton* and culturing the pulp of its organs (Figs. 3 to 11, plate 299).

On the cultures, especially on those of lung pulp, green colonies of *Penicilium* develop side by side, and often milk-white colonies of *Trichophyton;* the green and white colonies appear identical and differ only in their color; it is easy to distinguish, by their appearance, that the white colonies are colonies of *Trichophyton gypseum asteroides*.

If the spores of green *Penicilium* colonies are inoculated onto agar (especially unsweetened agar), green *Penicilium* colonies and white *Trichophyton* colonies quite often develop side by side; sometimes even half of a white colony turns green, transforming into *Penicilium.*

In the white colonies, more or less developed *Penicilium* fruiting bodies are observed (Figs. 2 to 8), and in the green colonies, the gonidial form is fully developed (Figs. 9, 10, 11). The *Penicilium* form is identified with that of the organic mold of the potato.

Trichophyton violaceum

The *Penicilium* form was obtained (pl. 300) by inoculating the guinea pig with *Trichophyton* and culturing the organs.

Achorion violaceum is a form of *Trichophyton violaceum*; transformed by passage through the guinea pig's organism, it gives the same *Penicilium* form. This is that of the organic mold of the potato.

Trichophyton lacticolor

The *Penicilium* form, obtained (pl. 301) by inoculating a guinea pig with *Trichophyton* and culturing its organs, is identical to that of the organic mold of the potato.

Trichophyton crateriforme

The *Penicilium* form (Figs. 2 to 7, pl. 301 bis), obtained by culturing the organs of the guinea pig inoculated with *Trichophyton*, is identical to that of the organic mold of the potato.

The culture of *Trichophyton*, inoculated on egg yolk, gave rise to a mold of the type *Botrytis cinerea* (Fig. 1, pl. 301 bis) and which is *Peronospora solani* or *Botrytis solani.*

I believed, after examination, that this mold was Bremia lactucae, with which it presents certain analogies; if this determination had been correct, it would have meant that the *Trichophyton* culture used was impure; but, later, I obtained this type of mold in cultures of the pulp of the organs of guinea pigs inoculated with potato tuber pulp. This fact therefore confirmed the origin of *Trichophyton crateriforme*; it is a vegetative form of the organic mold of the potato.

Trichophyton acuminatum

The *Penicilium* that I obtained (Plate 302) by culturing the organs of a guinea pig inoculated with *Trichophyton* has not been sufficiently cultivated and developed to allow identification. However, the types contained in Plate 302 are all found in cultures of *Penicilium* from the potato.

On the other hand, the culture of the monosterigmat gonidial type from the organic moiety of the potato has exactly the same acuminate form as the culture of *Trichophyton acuminatum;* this indicates that the latter must probably be the monosterigmat type of the potato converted to the *Trichophyton* form by cultivation on poor media.

Achorion Schoenleinii

By culturing *Achorion schoenleinii* on carrots, I obtained, by direct transformation, an ochre-colored mold presenting the evolved *Monosterigmata* form (Fig. 1, 2, pl. 303).

By inoculating the guinea pig with the *Achorion* and culturing the organ pulp, I obtai - ned the *Penicilium* form. The latter is identical to that of the organic mold of the potato.

The *Monosterigmata* form is also that of the potato, because it is obtained identically by culturing the organ pulp of guinea pigs inoculated with tuber juice, and, moreover, inoculating the guinea pig with this form makes it possible to obtain the *Penicilium* form of the potato, which is also given by direct inoculation of the *Achorion.*

The parasitic agent that causes favus is therefore potato organic mold, which is also vaccine mold.

Does favus result from a localized vegetative growth of vaccine mold that persists in the body as a permanent mycosis after vaccination, or is it due to a new infection or inoculation by potato organic mold?

The distinction seems of little importance, because it is certain that, due to the nature of the favus lesions, the pathogenic mold infects the entire organism.

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Let us point out here that the mouse, which is the animal most frequently affected by favus, is a rodent that eats ground meat, either in the ground or in dwellings.

Let us note, moreover, that the pits of favus on the scalp have so much the appearance of small smallpox pustules deprived of their scab that this disease could be called chronic smallpox of the scalp.

The same remark applies to favus of the body; the favian pits are slowly evolving smallpox pustules.

From these facts, it follows that favus is one of the distant manifestations of vaccinal or variola mycosis; it is chronic smallpox or vaccinia.

Other demonstrations support these conclusions. The different species of *Achorion* described so far, mainly *Achorion gypseum* and *Achorion Quinckeanum*, are established only on characters of low value and are confused with *Achorion Schoenleinii;* we will demonstrate that *Achorion gypseum*, which appears to be the most distinct from it, is also a vegetative form of the organic mold of the potato.

Achorion Gypseum

The form of *Achorion gypseum* is known (Figs. 1 to 5, pl. 304). I obtained its direct transformation into *Penicilium* in cultures on sugar agar. One colony transformed, in its lower half, into the green *Penicilium* form; it had a white upper half, another green half, and between these two parts a greenish-yellow zone. These three parts contained the stages of the transformation of *Achorion* into *Penicilium*; the white part contained the usual fruiting bodies of *Achorion* (Figs. 1 to 5, pl. 304); the greenish-yellow intermediate part contained fruiting bodies passing to the *Penicilium* type (Figs. 7, 9); the fruiting bodies are sacs in which the groups of sterigmata of *Penicilium* are formed Figure 9 shows a gonidiophore carrying at the same time, in A a sac still intact, in B, a ruptured sac which allows the sterigmata to pass through and, in C, a group of sterigmata completely liberated. In Figure 7, we see a sac A of characteristic shape still partly covering a group of sterigmata beside which there are two other groups already completely liberated. The green portion of the colony contained only the perfect form of the *Penicilium* represented in Figures 10, 11, 12. There can therefore remain no doubt that the *Penicilium* form is indeed an evolutionary form of the primitive type of *Achorion.*

I obtained the same *Penicilium* form by inoculating the guinea pig with *Achorion* and culturing the pulp of its organs. This *Penicilium* form is that of the organic mold of the potato.

The gonidial form of *Achorion gypseum* itself is also one of the gonidial forms of the organic mold of the potato. I have, in fact, isolated this gonidial form represented in Figures 8, 9, 10, Plate 146, by culturing on agar the central tissue of the potato stem reduced to pulp. To this gonidial form must be attached *Corticium solani* and *Alternaria solani,* which are only varied types. We also know that *Rhizoctonia solani* is a vegetative form of *Corticium solani.* A vegetative form of *Corticium violaceum*, which also grows on diseased potatoes and is surely a vegetative form of its organic mold, has been designated by the name *Rhizoctonia violacea.*

These explanations are provided to show how *Achorion schoenleinii, Achorion gypseum*, and *Achorion violaceum* can be vegetative forms that are in reality very little different from the same mold, that which constitutes the parenchyma of the potato.

Thus, *Achorion gypseum*, whose presence has been noted in certain favus, is, like *Achorion schoenleinii*, a vegetative form of the organic mold of the potato.

We have already noted above that *Trichophyton gypseum asteroïdes*, *lacticolor, crateriforme, violaceum*, and probably also *Trichophyton accuminatum*, are also vegetative forms of the organic mold of the potato. I believe that *Trichophyton radiolatum, Trichophyton granulosum*, and *Trichophyton vinosum* are also other forms.

Achorion violaceum

It has been previously stated that inoculating guinea pigs with *Achorion violaceum* and *Trichophyton violaceum*, followed by organ culture, gives rise to the same form of *Penicilium* (Pl. 30) and that this is the organic mold of the potato.

Epidermophyton inguinale

This mold, being cultivated on egg yolk, underwent a first transformation which increased its richness in fruiting bodies and brought them to a stable, characteristic form.

By inoculation of the guinea pig and culture of its organs, I obtained the *Penicilium* form (Fig. 3 to 8, pl. 305). The identification of this mold is under study.

The study of *Trichophyton* leads us to an important observation relating to the mycoses of the organism.

We have seen that most ringworms, at least the most frequent, are due to the growth of organic mold on the potato: this is also the case for ringworms attributed to *Trichophyton gypseum asteroides, lacticolor, violaceum, crateriforme*, and probably also to *Trichophyton accuminatum*.

Are these ringworms caused by external contamination by organic potato mold? Experience shows that this could be the case. Indeed, when the shaved and slightly scarified skin of a rabbit's back is infected with organic potato mold (bacterial cultures of various gonidial forms or tuber pulp), *the hair does not grow back for several months;* the skin remains completely bare and smooth.

But other facts indicate that ringworm of the scalp must probably come, in the vast majority of cases, from a cause other than external contamination. Indeed: *Trichophyton crateriforme* causes another lesion in other parts of the body, ringworm, which can exist alone, without the coexistence of ringworm of the scalp. This fact therefore establishes that contamination by organic potato mold has an internal origin; this contamination is the one conferred on the body by smallpox vaccination and smallpox. Ringworm is therefore one of the late manifestations of vaccinal or smallpox mycosis. It is very likely that common ringworm of the scalp has the same origin.

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It has been demonstrated above that *Achorion* are also vegetative forms of organic potato mold and that Favus can only arise, due to the nature of the lesions, from a general contamination of the organism; this contamination was caused by smallpox or smallpox vaccine, the pathogenic agent of which is a gonidial form of organic potato mold. Favus is a chronic cutaneous manifestation of smallpox or vaccinia. It is probable that trichophytosis of the plantar and trichophytic onychosis have the same origin.

These facts constitute examples of the late manifestations of long-lasting mycoses, mainly vaccinal mycosis.

It is likely that a more complete study of *Trichophyton* will show that some of them are forms of the molds that cause scarlet fever, chickenpox, etc.

While conducting a search for the isolation of scalp molds on a series of children consulting in the department of Doctor Sabouraut at Saint-Louis Hospital, I obtained, from the diseased scabs and hair of a young ten-year-old boy suffering from microscopy, the *Aspergillus fumigatus* form of organic oat mold, *that is, a gonidial form of the pathogenic mold of exanthematic typhus.*

In closing, I believe it is necessary to draw attention to the value of the characteristics that have served to distinguish the various species of *Trichophyton* described so far.

These characteristics are:

1. The shape of the colonies.
This form is so variable, for the same species, that I have completely abandoned its use; it is only in very rare cases that it can provide useful information. The same mold occurs in several forms, and these vary with the culture media and the number of generations.

This fact cannot be in doubt since a whole series of *Trichophyton* and *Achorion* are varied forms of the same species, the organic mold of the potato, the original source of the agents of vaccinia and smallpox.

Certain forms that were believed to be characteristic belong to a large number of very different species; the types of acuminate or crateriform colonies, radiant, etc., may belong to very distant species.

2. The color of the colonies.
Trichophyton lacticolor and *Trichophyton violaceum* are two forms of the same species, potato mold; color is therefore not enough to establish a specific difference between two molds. The same *Penicillium* culture can develop in both olive green and white green colors.

3. The shape of the mycelium and the arrangement of the spores or gonidia along the branches.
These two characteristics are so variable that they cannot be used to distinguish between neighboring forms or even between legitimate neighboring species.

From these explanations, it must be concluded that the fact of obtaining a form of white mold in acuminate or crateriform colonies does not mean that we are in the presence of the type that has been studied here; it may belong to other molds; in other words, a series of *Trichophyton* of different origins all appearing to belong to the same acuminatum type, for example, may, in reality, correspond to the organic mold of different plants. We can therefore only characterize a pathogenic mold of the scalp or a skin lesion by searching for its various gonidian forms and by identifying them, that is, by determining the plant or animal species that is the original source of the mold.

END